Concise Review on the Mechanisms and Clinical Utilities of Mesenchymal Stem and Progenitor Cells in Regenerative Medicine

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Abstract

Mesenchymal Stem Cells (MSCs) are multi-potent stem cells with multi-directional differentiation potential. The term MSPCs refers to mesenchymal stem and progenitor cells, which may exhibit full stem cell functions. MSPCs can be obtained from many tissue sources, such as adipose tissue, umbilical cord and bone marrow, etc. Studies show that MSPCs reside in the perivascular niche that is proximal to blood vessels. MSPCs are also capable of exerting their potential of homing and migration across the endothelium barrier toward lesion sites for repairing or regeneration. MSPCs can be stimulated to release a broad spectrum of bioactive factors including inhibitory factors and growth factors, and also express certain surface molecules, actions of which are relevant to a wide range of clinical applications such as the treatment of arthritis and diabetes mellitus. This review provides details of how MSPCs and MSPC-derived factors can potentially be used for homing and repairing mechanisms, and ultimately be applied in clinical settings.

Keywords: Mesenchymal Stem and Progenitor Cells, MSCs, Homing, Immunomodulation, Clinical Applications, Cell Therapy, Regenerative Medicine

Abbreviations: APC: Antigen presenting cell; BAX: bcl-2-like protein 4, is a protein that in humans is encoded by the BAX gene; BMP: Bone morphogenetic protein; CCR: C-C chemokine receptor; CCL: C-C ligand; C1P: Ceramide-1-phosphate; CXCL: Chemokine (C-X-C motif) ligand; CXCR: Chemokine (C-X-C motif) receptor; CC: Complement cascade; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; EGF: Epidermal growth factor; ECM: Extracellular matrix; ERK: Extracellular signal–regulated kinases; FGF: Fibroblast growth factor; TFH: Follicular helper T cell; GLUT4: Glucose transporter type 4; GTPase: Hydrolyze guanosine-5'-triphosphate; HCELL: Hematopoietic cell E- and L-selectin ligand, a specialized glycoform of CD44; HO-1: Heme oxygenase 1; HGF: Hepatocyte growth factor; HLA: Human leukocyte antigen; HA: Hyaluronic acid; IDO: Indoleamine-pyrroline 2,3-dioxygenase; IGF: Insulin growth factor; IPC: Insulin producing cell; IRS: Insulin receptor substrate; ILK: Integrin-linked kinase; IFN: Interferon; IL: Interleukin; IL1RA: Interleukin 1 receptor antagonist; FROUNT: Intracellular adapter; ICAM-1: Intracellular adhesion molecule-1; LPS: Lipopolysaccharide; M: Macrophage; MIP-1α: Macrophage inflammatory protein (CCL3-α); MHC: Major histocompatibility complex; MMP: Matrix metalloprotease; MSPC: Mesenchymal stem and progenitor cell; MSC: Mesenchymal stem cell; MAPK: Mitogen activated protein kinase; NK: Natural killer; NO: Nitric oxide; NKG2D: NK...
Introduction

In human fertilization, an ovum and a haploid sperm combine to form a fertilized egg that creates a single totipotential cell. Pluripotent stem cells from the inner mass of blastocysts can be isolated from cultures using specialized media, leading to differentiation into various cell lineages, such as hematopoietic stem cells (HSCs), neural stem cells and mesenchymal stem cells (MSCs). MSCs are characterized morphologically as long, thin and reticular fibrils-like cells and molecularly defined by the presence of stemness DNA signatures and specific markers. MSCs and their immediate progenitor cells collectively known as MSPCs, which can be isolated from Wharton’s jelly, for instance, exhibit a mesenchymal-like shape with a flat polygonal morphology [1]. MSPCs could adhere to the surface of tissue culture flasks. MSPCs are non-hematopoietic stem cells derived from mesoderm, have multi-directional differentiation potential, and consequently, they can differentiate into tissue-specific cells such as osteocytes, chondrocytes, adipocytes, hepatocytes, muscle cells, etc. MSCs are identified to exhibit low immunogenicity and high immune modulatory properties partially attributed to low expression of human leukocyte antigen (HLA) class I, lack of expression of HLA class II in general, and the expression of HLA-G [2,3]. Despite the evolving definitions, MSCs are currently identified by variable markers such as positive markers on CD73, CD90, CD105 and negative markers on CD11b, CD14, CD34, CD45, CD79α, and human leukocyte antigen-DR isotype (HLA-DR).

- Must have the capacity to differentiate into osteoblasts, adipocytes and chondroblasts in vitro.

Table 1: The basic and most common cell surface markers of MSCs [1]. Sca-1, stem cells antigen-1.

| Positive Markers | Stro-1, CD13, CD29, CD44, CD73, CD90, CD105, CD106 |
| Negative Markers | CD11b, CD31, CD34, CD45, CD117 |
| Variable Markers | Sca-1, Flk-1, CD10 |

The heterogeneous populations with different degrees of “stemness” and only some MSPCs populations exhibit full SC function, including the capacity of long-term self-renewal. Therefore, we will use MSPCs thereafter in this article to specify their functions and characteristics [7,8].

Studies indicate that MSPCs share the same origin and in vivo identity as pericytes and adventitial cells [9,10], which is relevant to their response to injuries and migration to lesion sites. MSPCs appear to offer several advantages for clinical applications due to their immune evasive and immune modulatory properties that greatly reduce the risk of immune rejection and graft-versus-host disease (GvHD). While other types of stem cells such as induced pluripotent stem cells (iPSCs) [11], embryonic stem cells (ESCs) [12], and hematopoietic stem cells (HSCs) [13] and so on are also under heavy investigation, MSPCs are among the leading cell types in clinical trials to date with their established safety and
efficacy profiles [14,15]. iPSCs involves the use of viral vectors for genetic reprogramming, hence the concerns over safety and genomic stability [16]. The access to ESCs requires embryo destruction and elicits strong ethical debates. Immune rejection and availability also severely limit the application of ESCs [17]. HSCs, while successfully used in bone marrow transplantation, are usually difficult to harvest in large quantities with CD34+ selection and ex vivo expansion [18]. Each cell type may face unique challenges in translation and targeted indications. On the other hand, one should not neglect the complex nature of MSPCs, and the development of MSPC-based therapy has to address the aspects including reproducibility of MSPCs as medical products, tumorigenicity, immune responses, short retention after implantation, altered cellular behaviors and differentiation into undesired tissues in vivo, and optimization of doses and route of administration [19].

In vitro MSPC culture and media

In order to reach a dose of MSPCs suitable for effective clinical applications in regenerative medicine, there is generally the need to go through a process of ex vivo expansion of the cells. These cultures should be conducted under conditions meeting good manufacturing practice principles and be demonstrated to not alter the characteristics of the expanded cells. Most procedures for the ex vivo expansion of MSPCs have used a standard growth medium (such as Minimal Essential Medium [MEM] or Dulbecco’s Minimal Essential Medium [DMEM]) supplemented with a source of nutrients, hormones or growth factors by adding fetal bovine serum (FBS) [20], typically at a final concentration of 10-15% (v/v). In the last few years, alternatives to FBS has been actively pursued due to immunological and infectious concerns linked to the use of biological materials from bovine origin [21]. Residual or MSPC-internalized antigenic bovine

Table 2: Specific surface markers of mesenchymal stem cells in various tissues [2].

<table>
<thead>
<tr>
<th>Markers Tissue Source</th>
<th>Positive Markers</th>
<th>Negative Markers</th>
</tr>
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<tbody>
<tr>
<td>Amniotic fluid</td>
<td>CD29, CD44, CD90, CD105, SH2, SH3</td>
<td>CD10, CD14, CD34, HLA-DR</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>CD73, CD90, CD105, STRO-1</td>
<td>CD45, CD14, CD34, HLA-DR</td>
</tr>
<tr>
<td>Gingival tissue</td>
<td>CD29, CD44, CD90, CD105</td>
<td>CD45, CD14, CD34</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>CD13, CD29, CD44, CD71, CD73, CD90, CD105, CD106, STRO-1</td>
<td>CD45, CD14, CD34, CD31</td>
</tr>
<tr>
<td>Dermis</td>
<td>CD44, CD73, CD90, CD105, SSEA-4, Vimentin</td>
<td>CD45, CD34, HLA-DR</td>
</tr>
<tr>
<td>Placenta</td>
<td>CD29, CD73, CD90, CD105</td>
<td>CD45, CD34</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>CD73, CD90, CD105</td>
<td>CD45, CD14, CD34, CD79, HLA-DR</td>
</tr>
<tr>
<td>Wharton’s jelly</td>
<td>CD73, CD90, CD105</td>
<td>CD45, CD14, CD34, CD79, HLA-DR</td>
</tr>
<tr>
<td>Umbilical cord blood</td>
<td>CD29, CD44, CD51, CD73, CD105, CD166, HLA-ABC, SH2, SH3, SH4</td>
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HLA = Human leukocyte antigen.

Table 3: Ligand/Receptor corresponding pairs and their sources [35,50].

<table>
<thead>
<tr>
<th>Ligand Source</th>
<th>Receptor</th>
<th>Receptor Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cells/inflammatory site(?)</td>
<td>CXCR4</td>
<td>MSPCs</td>
</tr>
<tr>
<td>MSPCs</td>
<td>P-selectin</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>MSPCs</td>
<td>E-selectin</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>VLA-4</td>
<td>MSPCs</td>
</tr>
<tr>
<td>MSPCs</td>
<td>CD44</td>
<td>MSPCs</td>
</tr>
<tr>
<td>Inflammatory site</td>
<td>CCR1</td>
<td>MSPCs</td>
</tr>
<tr>
<td>MSPCs</td>
<td>c-Met</td>
<td>MSPCs</td>
</tr>
</tbody>
</table>

SDF-1, stromal derived factor-1 (CXCL12); CXCR-4, chemokine (C-X-C motif) receptor-4; HCELL, hematopoietic cell E- and L-selectin ligand, a specialized glycoform of CD44; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4; HA, hyaluronic acid; MIP-1α, macrophage inflammatory protein (CCL3)-1α; HGF, hepatocyte growth factor; CCR1, C-C chemokine receptor; c-Met, tyrosine-protein kinase Met.
proteins could lead to the risk of xeno-immunization. In addition, bovine materials can be contaminated by various pathogens such as viruses and prions, potentially transmissible to human patients in spite of the species-barrier. These risks have stimulated the search for convenient alternatives [22], in a regulatory context where the use of materials from bovine origin tends to be discouraged when safe alternatives are available [23-25]. The use of chemically defined growth media has proven possible to culture established cell lines but remains difficult when it comes to growing primary cells which are physiologically and genetically more variable. In recent years, pooled human platelet lysates (HPL) made from clinical-grade platelet concentrates for transfusion have emerged as a reliable alternative to FBS [22,26-29]. Numerous studies have demonstrated that HPL added at a final concentration of 5-10% (v/v) is as effective as, if not more than, FBS to expand MSPCs. It allows one to propagate cells faster, maintain clonogenicity, and increase the CFU-F (colony-forming unit-fibroblast) size, while maintaining their immunophenotype and genetic stability, immune-suppressive effect in vitro, and their capacity to differentiate into the three main lineages (osteocytes, chondrocytes, and adipocytes) [22]. One additional advantage of pooled HPL is to provide a more reliable and consistent source of platelet growth factors than FBS [22]. While there are still questions unresolved with regards to the optimal production methods of HPL and their virus inactivation [30-33], its availability as a supplement of growth media may support the expected development of the use of MPSCs in regenerative medicine and advanced cell therapy.

Additionally, there is potential for allogeneic MSPCs to serve as an “off-the-shelf” therapeutic option, since these cells may be isolated and cultured from healthy donors in advance. This is in contrast to autologous MSPSCs, which need to be prepared per individual and may not be suitable or available for some acute conditions. Previously, all MSPCs were regarded as having low immunogenicity, since they all expressed only low levels of MHC class I and II, and exerted immunosuppressive functions in vitro and in vivo. However, recent studies have suggested that allogeneic MSPCs may not be as immune privileged as previously suggested. Schu et al. showed in rats that allogeneic MSPCs induced alloantibodies with the potential to activate complement-mediated lysis in vivo [34]. Intrastriatal administration of allogeneic MSPCs elicited a cellular immune response; this was, however, not strong enough to clear the administered cells [35]. Moreover, differentiation may elicit immunogenicity by increasing expression of MHC I and MHC II, and stimulating lymphocyte proliferation [36]. Allogeneic MSPCs are also able to educate CD8+ T cells with cytotoxicity against themselves. Interestingly, bone marrow-MSPCs elicited a higher level of lysis than adipose MSPCs [36]. It seems that different factors such as the disease model, route of administration, and experimental species have some effects on the immunogenicity of allogeneic MSPCs in vivo [37]. Interestingly, a randomized clinical trial comparing allogeneic MSPCs and autologous MSPCs for treatment of ischemic cardiomyopathy showed low immunogenicity of allogeneic MSPCs in addition to comparable efficacies of both cell populations [38]. On the other hand, autologous bone marrow-MSPCs were found to have better efficacy in improving acute kidney injury [39]. Nevertheless, both autologous and allogeneic MSPCs have been applied in transplantation studies with promising results [40-42]. Considering there still exist such equivocal results, more comparative studies on the potential clinical applications with allogeneic vs. autologous MSCs are warranted.

Factor-releasing properties

MSPCs possess factor-releasing properties that play a vital role in MSPC-based therapy. Inflammatory chemokines released by inflammatory sites recruit T cells and antigen presenting cells (APCs) to lesion sites [43,44]. During an immune response, inflammatory cytokines produced by T cells and APCs modulate the function of MSPCs leading to the secretion of growth factors, inhibitory factors and the expression of certain surface molecules on the MSPC membrane. Activation of MSPCs by inflammatory cytokines is an inflammatory cytokine licensing process of MSPCs [45]. Licensed MSPCs homing toward a lesion site is mainly governed by the surface molecules such as VLA-4 (very late antigen-4), CXCR4 (CXC-receptor-4) that are involved in capturing, rolling, firm adhesion and transendothelial migration. Growth factors secreted by MSPCs may promote repairing and regeneration of damaged sites in certain organs or tissues. Usually a specific group of growth factors are in favor of creating a trophic microenvironment surrounding the damaged tissue site for regeneration. MSPCs and the cells of immune system interact closely. MSPC-derived inhibitory factors such as prostaglandin E-2 (PGE2) and indoleamine-pyrrole 2,3-dioxygenase (IDO) can inhibit NK cells and cytotoxic T cells, and also modulate regulatory T cells (Treg) function in general (Figure 1). Of note is that apart from the MSPCs derived factors, cell-cell interactions among MSPCs and immune cells and stromal cells, where surface molecules come to play within the injured lesion, are also important in the cell migratory and regenerative processes [43,45].
Homing mechanism

MSPCs possess intrinsic homing ability to migrate to injured tissues and actively participate in tissue repairing [46]. Previous studies revealed MSPCs homing mechanism by investigating and comparing with the leucocyte extravasation mechanism [47]. The mechanisms of MSPCs and leukocytes homing may share some similarities. When tissue damaging occurs, MSPCs either in the immediate vicinity of the lesion sites or those derived from the bone marrow are believed to migrate toward the damaged tissue [48]. On the basis of MSPCs obtained from various tissues and their homing ability, a perivascular niche has been identified. A perivascular niche is in close proximity to sinusoid in the extracellular matrix, illustrating the reason why MSPCs are able to be derived from various tissues and vascularised sites such as bone marrow [49]. It is suggested that MSPCs migrate to a lesion site through a sequence of events.

First, MSPCs are activated by inflammatory cytokines which tend to be licensed MSPCs and migrate via blood circulation reaching aspecific lesion site. Second, MSPCs are captured by the endothelium and rolling on the endothelium wall, which is primarily mediated by selectins. Third, firm adhesion between MSPCs and endothelium are mediated by a series of ligand/receptor pairs, especially integrins. Firm adhesion leads MSPCs to crawl on the endothelium wall and scan for weak points to overcome the endothelium barriers. After searching for exit cues, MSPCs are polarized by certain regulators and overcome the endothelium barriers, basal lamina and pericytes before continuing inflammatory cytokine-guided interstitial migration to eventually enter the lesion sites [47,48].

Previous studies of MSPCs homing mechanisms are largely based on MSPCs prepared from exogenous sources. The mechanisms of MSPCs migration still remain to be elucidated; however, there is currently no evidence that indicates differences in migration toward a lesion site between autologous MSPCs and allogeneic MSPCs [47]. The homing mechanisms of MSPCs toward injured sites will be discussed step by step in the following sections (Figure 2).

Figure 1: Under stimulation by inflammatory cytokines, licensed MSPCs secrete a spectrum of factors such as surface molecules, growth factors and inhibitory factors (modified after Katsuda et al., Figure 1 [43]). Surface molecules play an essential role in homing of MSPCs to inflammatory sites. Growth factors may create a trophic microenvironment for tissue regeneration. Inhibitory factors possess immunomodulation properties on cells of the immune system. APC, antigen presenting cell; MSPC, mesenchymal stem and progenitor cell; TEM, transendothelial migration; ECM, extracellular matrix; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; NO, nitric oxide; IDO, indoleamine-pyrrole 2,3-dioxygenase; PGE-2, prostaglandin E-2.
Activation: T cells and APCs from inflammatory sites release inflammatory cytokines (e.g. interferon-γ, IFNγ, and tumor necrosis factor-α, TNFα) [47]. Inflammatory cytokines act as signals in order to induce and modulate MSPCs functions. MSPCs activated by inflammatory cytokines tend to be licensed MSPCs that have the capacity of homing and repairing inflammatory tissues. Licensed MSPCs may "sense" inflammatory sites by inflammatory cytokines and express surface molecules on its membrane preparing for further actions with the endothelium. Endothelial cells that contribute to homing are activated by upregulation of receptors on the endothelium in response to mediators released by inflamed tissue. Those mediators such as TNFα induce activation of P-selectins on the endothelial cells surface within a few minutes, increase E-selectins expression and reach peaks after 3-4 hours. Vascular cell adhesion molecule 1 (VCAM-1) expressed by the endothelium is also stimulated by inflammatory cytokines from the activation of intracellular signaling pathways [47]. MSPC-expressed receptor very late antigen-4 (VLA-4) interacts with corresponding ligand VCAM-1. Inflammatory cytokines upregulate VCAM-1 and activate VLA-4, leading to initial arrest of MSPCs on the endothelium surface. The chemokine stromal cell-derived factor 1 (SDF-1, also known as CXCL12) is critical for MSPCs chemotaxis and homing in response to inflammatory cytokines through interactions with CXCR4 on the MSPC surface [50].

Capture: Licensed MSPCs are shown to express ligands such as galectin-1/glycoprotein and HCELL that bind to corresponding receptors, i.e. P-selectins and E-selectins, expressed by endothelium, respectively. These two pairs of ligand/receptor initiate the capture
of MSPCs to endothelium and mediate further rolling on the endothelium. Platelet bridging is pointed out to assist with P-selectins in capturing licensed MSPCs [47,48].

**Rolling:** MSPCs rolling is obligatory and is facilitated by galectin-1 binding with P-selectins or platelet bridging that act as alternatives for selectin-mediated adhesion. Besides selectins that mediate rolling, integrin VLA-4 is also an important mediator of endothelial rolling and arrest at the sites of inflammation. Due to MSPCs that could be passively arrested or slowed down, rolling of MSPCs may not be crucial for establishing firm adhesion [47].

**Firm adhesion:** Activation of surface molecules to mediate firm adhesion between MSPCs and the endothelium is a key mechanism of the homing process. The expression of numerous ligands and receptors is shown to contribute to firm adhesion including HA/CD44, MIP-1α/CCR1, HGF/c-Met, SDF-1/CXCR4 (ref. to Abbreviation list) and most importantly VCAM-1/VLA-4 (Table 3). The key ligand/receptor pair VCAM-1/VLA-4 establishes firm adhesion, mediated via the Rac1/Raq1 GTPase signaling. The HCELL binding to E-selectin affects VCAM-1/VLA-4 adhesion and triggers activation of G-protein signaling, while bypassing chemokine receptor signaling. MIP-1α is a chemokine that causes migration of pro-inflammatory cells to sites of inflammation and regulates their transendothelial migration. MIP-1α expression is induced by inflammatory stimuli. MSPCs express CCR1 to interact with MIP-1α [51]. MIP-1α may increase homing of MSPCs to injured sites. However, the chemotaxis of MIP-1α still needs to be identified. Moreover, the concentration of c-Met is upregulated in wound areas and shown to act as a chemoattractant for MSPCs [52].

**Crawling & scanning:** Firm adhesion results in licensed MSPCs crawling on the endothelium and form filopodia by stimulation with CXCL9 (CXCL9 is a CXC-motif, T-cell chemoattractant, induced by the inflammatory cytokine IFN-γ). During crawling, MSPCs scan endothelium searching for exit cues to overcome endothelium barrier. In *in vitro* live cell imaging studies on transmigrating MSPCs, blebs have been suggested to mediate inter-cellular forces against endothelium. Also MSPCs and endothelium actively cooperate to enable guidance by trans-migratory cups, a form of endothelial protrusions [47].

**Polarization:** MSPCs require polarization before transendothelial migration. MSPCs show little lateral migration on the endothelium during polarization. The intracellular adapter molecule FROUNT links to CCR2, followed by CCR2 clustering, which leads to cytoskeletal reorganization. FROUNT was required for polarization of MSPCs, resulting in clustering of CCR2 and reorganization of the cytoskeleton [47,53]. Cytoskeletal remodeling facilitates opening of endothelial junctions and eases the passage of the transmigrating cells.

**Transmigration:** MSPCs have to overcome three barriers: endothelium, basement membrane (basal lamina) and pericyte sheath during transendothelial migration in general.

**Overcome endothelium**

MSPCs advance against the endothelial barrier by the formation of bleb-like protrusions (cup-like structures) on the cell surface, particularly at sites of close contact with the endothelium. MSPCs overcome the endothelial monolayer also by cooperating with endothelial cells. Only a few signaling pathways related to transendothelial migration (TEM) have been identified so far. For example, phosphokinase C (PKC) is upregulated in an IL-8 dependent manner in human MSPCs. MSPCs TEM dependent on phosphoinositide 3-kinase (PI3K)/Akt and Rho kinase (ROCK) [47]. As integrins lack enzymatic activity, an intracellular adapter transduces signals from outside or from the inside, one intracellular adapter integrin-linked kinase (ILK) plays a role for MSPCs TEM and signaling. Rho is identified as a regulator of cytoskeletal activation and a modulator of MSPCs transmigration.

**Overcome basal lamina**

Basal lamina consists of collagen, lamin and fibronectin in general. As soon as the leading edge reaches the basal lamina, the matrix metalloprotease (MMPs) proteolytic enzymes may be synthesized and released upon inflammatorycytokinesstimulation[54]. Pro-inflammatory cytokines: IFN-γ and TNF-α increase production of MMPs in MSPCs, thereby enhancing the capacity of MSPCs to migrate through the extracellular matrix. MMPs are able to degrade basal lamina components collagen type IV and tissue inhibitor of metalloprotease 3 (TIMP-3) [54]. Urokinase-type plasminogen activator (PA) found in MSC protrusions can support the migration of MSPCs via ERK1,2 MAPK signaling.

**Overcome pericytes**

Pericytes are contractile cell layer that wrap around
the endothelial cells. ICAM-1 interplay regulates the passage through the pericyte sheath [47,55]. The chemo attractants may vary at different injury sites. Ceramide-1-phosphate (C1P) and sphingosine-1 phosphate (S1P) are bioactive lipids resisting degrading enzymes as chemotactic homing signals to MSPCs when SDF-1 is degraded by other enzymes surrounded inflammatory sites [50]. C1P and S1P bind with their corresponding receptors expressed by MSPCs [55,56]. MSPCs have been shown to interact with immune cells during inflammation, and these interactions may impact the way MSPCs contribute to tissue repairing. Complement is a major humoral component of the innate immune responses. The complement cascade activated by different pathways leads to the generation of bioactive peptides such as C3a and C5a that chemo-attract MSPCs [56].

**Repair mechanisms**

When a tissue is injured or damaged, MSPCs and cells of the immune system migrate from blood vessels across the extracellular matrix to reach the injured tissue sites by the stimulation of inflammatory cytokines. Since MSPCs have multi-directional differentiation potential, it is believed that MSPCs can differentiate into functional cells to replace damaged cells. Meanwhile, in response to inflammatory cytokines, MSPCs release both growth factors and inhibitory factors [57]. A large amount of growth factors are produced to subsequently stimulate MSPCs into endothelial progenitor cells, fibroblast cells and tissue progenitor cells or directly facilitate differentiation of the progenitor cells. The concerted actions of these factors and cells facilitate tissue repairing through angiogenesis, remodeling of the extracellular matrix (ECM), proliferation and differentiation of tissue progenitor cells for replacement. The inflammatory milieu, including pro-inflammatory cytokines, strongly inhibits MSPCs differentiation potential. Therefore, sufficient suppression of inflammation prior to the operation is important for efficient tissue regeneration. Inhibitory factors modulate the progression of inflammation by suppressing immune cells to cease from releasing inflammatory cytokines. Recent studies on MSPC-mediated immune regulation suggest that MSPCs are recruited to inflammatory sites and activated through inflammatory cytokines produced by activated immune cells such as T cells and APCs. MSPCs activation leads to the production of immune regulatory factors such as inhibitory factors and growth factors. Depending on the types of immune responses (acute or chronic inflammation), MSPCs may either attenuate the inflammatory response, and lead to repairing of the damaged tissue, or maintain a persistent chronic inflammatory response, leading to fibrosis and deformation of tissue architecture (Figure 3) [58].

During acute inflammation, MSPCs release both inhibitory factors and growth factors in response to inflammatory cytokines. Growth factors favor MSPCs differentiation into functional cells such as endothelial cells and fibroblasts, remodeling the surrounding inflammatory tissue structure. After the injured tissue is repaired, the inhibitory factors secreted by MSPCs inhibit cells of the immune system releasing inflammatory cytokines [59,60]. During chronic inflammation or acute inflammation remission, MSPCs are able to release low levels of both growth and inhibitory factors [59,60]. The inhibitory factors are not sufficient to inhibit cells of the immune system. The growth factors continue to assist MSPCs with differentiation into functional cells such as endothelial cells and fibroblasts for tissue structure formation under the stimulation by a low level of inflammatory cytokines released. Basically, MSCP-based therapy depends on two main properties of MSPCs; one is MSPC’s multi-directional differentiation potential, the other is the ability of MSPCs to release large amounts of factors in response to diverse stimulation, and perform different functions.

**Immunomodulation properties**

Studies have shown that the immunomodulation ability of MSPCs is not constitutive, instead, it is licensed by inflammatory cytokines in an inflammatory microenvironment [61]. MSPCs affect both innate immunity and adaptive immunity. Regarding innate immunity, MSPCs influence macrophages and NK cells. Macrophages are key effector cells in innate immunity and are involved in tissue defense, homeostasis and repairing [62]. They can exhibit either a pro-inflammatory or an anti-inflammatory phenotype depending on the microenvironment associated with the successive phases of the inflammatory response. MSPCs are able to polarize pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages phenotype through PGE2 interaction with EP2 and EP4 receptors on macrophages to induce secretion of IL-10. Moreover, MSPCs secret TSG-6 (TNF-α-stimulated gene-6 protein), which interacts through CD44 receptor on resident macrophages. The CD44 molecule is dissociated from TLR-2, leading to impairment of pro-inflammatory cytokines expression [61]. NK cells play a critical role in the defense against virus-infected cells and tumor cells. MSPCs influence NK cells pro-inflammatory cytokines production. For example, MSPCs inhibit IFN-γ secretion by IL-2 and/or IL-
15 activated NK cells. In addition, MSPCs impair NK cells cytotoxic activity through cell-cell contact and soluble factors such as IL-10, PGE$_2$, HLA-G5 and TGF-β to down-regulate the natural cytotoxicity receptors, NKp30 and NKp44, and the NK group 2D (NKG2D) [61].

For adaptive immunity, MSPCs interact with T cells, B cells and dendritic cells, respectively. T cells are the major cellular effector of the adaptive immune response and play a central role in cellular mediated immunity. T cells activation is dependent on binding of antigen-presenting MHC class I and non-antigen-specific co-stimulatory molecules including CD40, CD80 and CD86 expressed by donor cells to their ligands on T cells. There are three types of T cells, cytotoxic CD8+ T cells, CD4+ helper T cells and regulatory T cells [60]. All of them are implicated in immune responses in general. CD8+ cells are cytotoxic activated T lymphocytes. They can induce the killing of target cells. CD8+ T cells cytotoxicity can be inhibited by HLA-G5 secreted by MSPCs. CD4+ T cells are helper T cells, which can be induced by different stimuli to differentiate into Th1, Th2 and Th17 or regulatory T cells [60]. MSPCs can support inhibition of Th1 response, promote the secretion of IL-4 and the T cells phenotype shift to the anti-inflammatory Th2 phenotype [61]. Th17 cells secrete not only IL-17 but also IL-17F, IL-21 and IL-22; these cytokines most likely cooperate to induce tissue inflammation and Th17-driven effector functions may be different in different tissues [63]. Regulatory T cells (T$_{reg}$) comprise several specialized subsets of T cells that are able to control immune responses and promote or maintain immune tolerance in an antigen-specific way. MSPCs secret soluble factors such as PGE$_2$, TGF-β and HO-1 to induce three major T$_{reg}$ cells subsets corresponding to IL-10+ T regulatory 1 (Tr1), TGF-β+ T helper 3 (Th3) and CD25+FOXP3+ natural T$_{reg}$-like CD4+ cells [64]. These T$_{reg}$ cells are functional and can efficiently suppress T-cells proliferation triggered by antigenic peptides. MSPCs also induce a more anti-inflammatory T$_{reg}$ cells and promote T$_{reg}$ cells proliferation or regeneration [61,64]. MSPCs can support inhibition of Th1 response by decreasing pro-inflammatory cytokines (TNF-α, IFN-γ) and increasing IL-4. MSPCs also inhibit differentiation of naive T cells into Th17 cells by inducing the production of anti-inflammatory cytokines IL-10 and modulate transcription factors through cell-cell contact and soluble factors: PGE$_2$ and IL-10. Th17 and Th1 cells are both pro-inflammatory phenotypes playing important roles in inflammatory responses of the human body and have been shown to be involved in a variety of autoimmune diseases. MSPCs polarize CD4+ T cells into T$_{reg}$ cells and Th2 cells in a greater anti-inflammatory phenotype. B cells play a major role in humoral-mediated immunity. B cells differentiate into immunoglobulin-secreting plasmablasts after antigenic stimulation and are also potent APCs [65]. MSPCs increase B cells viability while also inhibit proliferation after polyclonal stimulation mimicking the three signals (B cells receptor engagement, costimulation and cytokine or toll-like receptor activation) of B cells activation [65]. MSPCs also inhibit B cells differentiation by decreasing CD38+/CD138+ expression after exposure to dendritic cells, and impaired antibody production [65]. MSPCs through cell-cell contact may also inhibit B cells proliferation and terminal differentiation into plasma cells through down-regulation of the transcription factors [61]. Regulatory B cells (B$_{reg}$) are a peculiar subset of B cells that can produce anti-inflammatory cytokine (IL-10). MSPCs increase the percentage of B$_{reg}$ secreting IL-10 [65]. Dendritic cells (DCs) are the most potent APCs that can initiate and regulate the adaptive immune responses by promoting antigen-specific T cells activation [60]. MSPCs strongly inhibit the initial differentiation of both CD34+ cells and monocytes into DCs. DCs generated in the presence of MSPCs fail to express pro-inflammatory cytokines (such as TNF-α and IL-12), class-II MHC and costimulatory molecules, but secrete large amounts of the anti-inflammatory cytokine IL-10 [61]. MSPCs inhibit the activation and function of both adaptive and innate immune cells in immune-modulation (Figure 4).

**Summary of secreted bioactive factors**

MSPCs possess the ability to secrete a broad range of bioactive factors under inflammatory cytokines stimulation. Generally, one single bioactive factor cannot accomplish one specific function [66]. A group of factors are suggested for a specific function during MSPC-based regeneration. When licensed MSPCs migrate to the damaged sites, large amounts of factors such as growth factors, inhibitory factors are released by the licensed MSPCs [67]. These factors have particular functions: chemo attraction, immunomodulation, anti-apoptosis, angiogenesis and anti-fibrosis in general. They co-work with each other to achieve the eventual regeneration of injured sites. The factors such as chemokines and surface molecules released by licensed MSPCs are in favor of MSPCs homing and migration to the damaged sites for regeneration. Homing ability of MSPCs is a fundamental property during tissue regeneration. MSPCs secreted factors possess immunomodulation ability to suppress T cells and B cells proliferation and function, inhibit CD8+ cells and NK cells cytotoxicity, inhibit differentiated Th1 and Th17 responses. MSPCs may also regulate T$_{reg}$ cells
function, polarize macrophage 1 pro-inflammation status, polarize DCs so that they fail to express pro-inflammatory cytokines, but secrete anti-inflammatory cytokines. The immunomodulation of MSPCs ultimately inhibits pro-inflammatory cytokines releasing and produces more anti-inflammatory cytokines on the lesion sites depending on the inflammatory tissue. MSPCs can secrete growth factors to create a trophic environment to achieve anti-apoptosis, angiogenesis and rebuild the surrounding ECM. Furthermore, MSPC-secreted factors can balance the concentration of proteolytic enzymes (MMPs) and tissue inhibitor MMPs. MMPs can degrade fibroblasts to achieve anti-fibrosis of damaged tissue. Tissue inhibitor MMPs are able to inhibit MMPs function.

**Clinical applications**

MSPCs are attracting an increasing amount of attention due to their unique therapeutic properties, mainly, homing capability, immune modulatory properties, and regenerative capacity. In order to illustrate how MSPC-derived bioactive factors translate to clinical applications more clearly, two disorders, arthritis and diabetes mellitus, will be discussed in this context. Both arthritis and diabetes mellitus have high incidences worldwide, especially among the elderly. The pathologies of arthritis and diabetes mellitus may share some similarities, for example, immune cells attacking the normal tissue cells and over-production of pro-inflammatory cytokines. Considering the wide ranging curative properties of MSPCs, one may surmise there exist common denominators in their therapeutic approach.

**Osteoarthritis**

Osteoarthritis is a progressive degenerative disease of multiple etiology in which injury and aging lead to gradual breakdown of articular cartilage [68,69]. The pathogenesis is categorized by severe inflammation, recruitment of inflammatory cells, high level of pro-inflammatory cytokines (TNF-α, IL-1, IL-6) production, synthesis and release of proteolytic enzymes (aggrecanases, MMPs) by the primary lesion sites [69]. The series of events leads to degradation of ECM and decreasing in the production of ECM components such as proteoglycan, ultimately leading to chondrocytes death and bone exposure. Of note, IL-1 and TNF-α are primary drivers of a cytokine led degradation of cartilage, both of them increase synthesis of MMPs and decrease MMPs enzyme inhibitors, resulting in a net catabolic environment and loss of extracellular matrix [70]. MMP-13 serves as a major mediator of type II collagen cleavage and matrix degradation.

MSPCs release growth factors to create a trophic environment for chondroprogenitors differentiation into normal chondrocytes [71]. The recruitment and proliferation of MSPCs is followed by differentiation and proliferation of chondroprogenitors, and their maturation into chondroblasts and eventually chondrocytes [68]. The newly generated chondrocytes are able to replace the dead or damaged chondrocytes. Also, MSPCs are able to release sufficient factors contributing to the processes of anti-inflammatory, anti-proteolytic enzymes and anti-apoptosis [72]. Upon stimulation of inflammatory cytokines, MSPCs produce more anti-inflammatory factors such as IL-10 and IDO, transform pro-inflammatory macrophages type 1 into anti-inflammatory macrophages type 2 directly and decrease expression of pro-inflammatory factors such as IL-1 and TNF-α. Because high levels of proteolytic enzymes (MMPs and aggrecanases) are synthesized and released by the primary lesion sites, degraded cartilages undergo ECM breakdown. MSPC-secreted factors modulate TIMP-1 & TIMP-2 inhibition of MMPs, and decrease expression of MMP-1 and MMP-13 levels directly. MSPCs may also secrete factors to inhibit chondrocytes death and cartilage loss through anti-apoptosis factors and decrease the level of apoptosis activator BAX [68,73]. BAX can stimulate chondrocytes apoptosis and bone exposure. It should be emphasized that MSPCs are able to secrete a board spectrum of factors to exert their specific functions depending on the microenvironment of tissue involved (Figure 5) [74].

**Rheumatoid arthritis**

Rheumatoid Arthritis (RA) is a chronic and systemic inflammatory disease, characterized by the destruction of the articular cartilage and bone in its chronic phase [73,75,76]. Synovium undergoes a pathological outgrowth, sustained by proliferation of synovial fibroblasts and infiltration of inflammatory or immune cells that invade and erode articular cartilage and bone, leading to deformity, pain and disability [77]. In RA, pro-inflammatory cytokines, such as TNF-α, IL-6, IL-17 and IL-1β, play dominant pathological roles. Pro-inflammatory cytokines induce cartilage destruction by disturbing metabolic balance through the suppression of cartilage matrix production, enhancement of production of cartilage matrix-degrading enzymes by chondrocytes, and induction of chondrocytes apoptosis [78]. The fibroblast-like synoviocytes are resident cells of synovial joints, involved in pannus formation, are key players in
Figure 3: Repair mechanism of MSPCs. Under stimulation, licensed MSPCs and cells of the immune system are mobilized towards the site of damaging to exert their functions (modified after Wang et al., Figure 1 [45]). MSPC, mesenchymal stem and progenitor cells.

Figure 4: Immunomodulation properties of MSPCs (modified after Abumaree et al., Figure 1 [61]). MSPCs may exert immunomodulation properties to modulate both innate (macrophages and NK cells) and adaptive immunities (T cells, B cells and DCs). Treg, regulatory T cell; NK, natural killer cell; DC, dendritic cell; PGE-2, prostaglandin E-2; TSG-6, tumor necrosis factor-inducible gene-6; IL, interleukin; TGF-β, transforming growth factor-β; IDO, indoleamine-pyroline 2,3-dioxygenase; HLA-G5, human leukocyte antigen-G5; HO-1, heme oxygenase 1.
the destruction of cartilage and bone in RA joints. Due to the limited regenerative ability of chondrocytes, defects in cartilage are irreversible and difficult to repair.

MSPCs modulate cells of the immune system and promote repairing for RA [68]. MSPCs can modulate T cells function, including suppression of T cells proliferation and activation of T<sub>reg</sub>. MSPCs can induce a regulatory phenotype in Th17 cells [77,79]. Increasing the proportion of Th17 cells expressed the anti-inflammatory cytokine IL-10 [80]. This mechanism is dependent on glucocorticoid-induced leucine zipper, which inhibits the pro-inflammatory transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells and activator protein, in MSPCs [81]. Follicular helper T cells (Tfh) provide help for antigen-specific B cells. It is shown that Tfh cells frequency were increased and associated with auto-antibodies in patients with rheumatoid arthritis, suggesting a possible involvement of Tfh cells in its pathogenesis [81]. MSPCs can suppress differentiation of Tfh cells in RA patients partly via the production of IDO.

In addition, MSPCs may inhibit dendritic cell maturation and polarize macrophages to an anti-inflammatory phenotype dampening inflammation and preventing joint damaging [79]. Osteoclasts play an important role in bone resorption leading to joint destruction in RA. MSPCs inhibit osteoclastogenesis by constitutive production of osteoprotegerin (OPG) and other inhibitory factors that are beneficial for the inhibition of bone erosion in RA. MSPCs also produce multiple factors including IL-6, which is one of the major humeral factors released from MSPCs [78]. Furthermore, IL-6 mediate signaling in MSPCs differentiation into chondrocytes. The inflammatory milieu, including pro-inflammatory cytokines, strongly inhibits MSPCs differentiation into chondrocytes. Therefore, sufficient suppression of articular inflammation prior to the operation is important for efficient cartilage regeneration while using MSPCs in cartilage regenerative therapy (Figure 6) [63,79].

**Type 1 diabetes mellitus**

Type 1 Diabetes Mellitus (T1DM) results from a combination of environmental risk factors, genetic predisposition and autoimmune destruction of insulin-producing β cells in the pancreas [82]. Transplantation of pancreas used to be a standard therapy; however, it has several limitations [83]. In the progression of T1DM, β cells auto antigens are recognized by antigen-presenting cells (APCs), including dendritic cells and macrophages, which are the first to infiltrate islets followed by T lymphocytes, NK cells and B cells. The autoantigens are present together with the MHC-II molecules on the surface of APCs to trigger autoimmune responses. These autoantibodies together with NK cells contribute to the destruction of pancreatic β cells via antibody-dependent cellular cytotoxicity [82]. Th1 secrete IL-2 and IFN-γ, which promote the cytotoxicity of CTLs, inhibit Th2 cells

![Figure 5: Mechanism of MSPCs treatment of osteoarthritis by its released factors (modified after Wang et al., Figure 1 [74]). Red boxes illustrate the pathology of osteoarthritis, and green boxes show how MSPCs treatment can efficiently counteract with each of those itemized in the pathology of the disease in a corresponding manner. BMP, BMP, bone morphogenetic proteins; FGF, fibroblast growth factor; TGF, transforming growth factor; IGF, insulin growth factor; SOX, transcription factor; IL, interleukin; IDO, indoleamine-pyrrole 2,3-dioxygenase; TIMP, tissue inhibitor MMP; MMP, matrix metalloprotease; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor.](image)
and weaken the protective effects of Th2 cells [84]. Th17 secrete IL-17 in response to β cells autoantigens, which attracts inflammatory cells infiltration and enhances neutrophils function. Macrophages type 1 (M1) are activated by Th1 secreted IFN-γ. Macrophages secrete IL-1 and TNF-α, induce structural changes of β cells and suppression of their insulin releasing capacity [82]. The cytotoxic activity of macrophages may also contribute to β cells destruction. Thus, Th1, Th17 and macrophage induce β cells apoptosis by secreting cytokines [84]. Therefore, β cells apoptosis results from a high level of pro-inflammatory cytokines. Meanwhile, Th2 cells and T\(_{\text{reg}}\)s are able to protect β cells by expressing anti-inflammatory cytokines. Recent studies have shown that MSPCs could be applied in the treatment of T1DM. MSPCs can promote the secretion of IL-4 and the T cells phenotype shift to the anti-inflammatory Th2 cells. Inhibition of Th17 differentiation and IL-17 production mediated by MSPCs also contribute to treatment with T1DM. MSPCs can reduce CTLs function via direct effects on CTL as well as through inhibition of Th cells responses. Also MSPCs are able to enhance T\(_{\text{reg}}\)s proliferation, which can secrete IL-10 and TGF-β, mediating the inflammatory reaction of Th1 and Th17 and reduce the cytotoxicity of CTLs [84]. MSPCs have the ability to modulate the phenotype of macrophages by inducing a shift from pro-inflammatory phenotypes to anti-inflammatory phenotypes. MSPCs may serve as trophic mediators to support islet functions. It has been shown that insulin producing cells can be regenerated from the transdifferentiation of other cells, pancreatic duct cells, and acinar cells. MSPCs secret several factors such as IL-6, VEGF-A, HGF and TGF-β, that seem to improve islet cells viability and function by inhibiting apoptosis, inducing β cells proliferation, enhancing β cells insulin response to high glucose, and promoting islet revascularization (Figure 7) [85].

**Type 2 diabetes mellitus**

Type 2 Diabetes Mellitus (T2DM) is characterized by the combination of relative insulin deficiency and insulin resistance. Since MSPCs possess differentiation potential, exhibit immunomodulation properties and anti-inflammatory effects, they are considered an ideal cell agent for the treatment of T2DM [82,86]. Their therapeutic actions may be summarized as follows. First, key transcription factor pathways are activated to induce differentiation of MSPCs into insulin producing cells (IPC) that provide typical β cell functions. The distinction between β cells and IPC is the physiological regulation of insulin secretion performance. In addition, IPCs could be derived from MSPCs, whereas β cells are considered as origination from endogenous pancreatic progenitor cells [86]. Second, MSPCs secreted factors allow endogenous cells to proliferate in order to promote regeneration of islet β cells. The paracrine factors include vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF)-BB, and angiopoietin-1. In addition, islet alpha cells have potential for reprogramming into β cells [86].
Third, MSPCs suppress immune cells proliferation and functions, because of low intracellular expression of class II major histocompatibility proteins and co-stimulatory molecules. Hence, MSPCs attenuate the autoimmunity that leads to the destruction of pancreatic β cells. MSPCs promote islet survival against hypoxia and oxidative stress. The oxidative stress injury induced by hyperglycemia is recognized as a major etiological factor in diabetes development. Autophagy is a common intracellular degradation process by which eukaryotes maintain intracellular homoeostasis via degradation and recycling of damaged organelles and toxic proteins. Basal autophagy is essential for maintaining the architecture and function of pancreatic islet β cells. Enhancement of autophagy may improve treatment of T2DM [86]. Fourth, MSPCs secreted factors ameliorate insulin resistance. Activation of the insulin receptor substrate (IRS-1) signaling pathway results in increased translocation and expression of GLUT-4 (glucose transporter type 4). GLUT4 facilitates the diffusion of circulating glucose down its concentration gradient into muscle and fat cells. Insulin resistance is now considered to be closely related to systemic chronic low-grade inflammation. M1 pro-inflammatory state polarization to anti-inflammatory subset M2 plays a role in preventing insulin resistance. The treatment of T2DM using MSPCs is therefore a potential therapy due to the factors-releasing properties of MSPCs [86].

**Conclusions**

MSPCs are promising candidates for cell therapy in regenerative medicine. One of the main benefits of MSPCs is their capability of secreting a broad spectrum of factors in response to inflammatory cytokines. Surface molecules expressed by MSPCs contribute to the homing process, especially in capturing, rolling, firm adhesion, and TEM between MSPCs and the endothelium. During
Repairing of injured or inflammatory tissues, inhibitory factors contribute mainly to the immune modulatory effects between MSPCs and immune cells. Growth factors released by MSPCs may create a trophic microenvironment for endogenous tissue progenitor cells which differentiate into normal endogenous tissue cells. This rebuilds the ECM and endothelium surrounding the lesion sites, ultimately achieving inflammation, remodeling and regeneration at the sites of injured tissue. This flexibility circumvents several difficulties in the current standard treatments with arthritis and diabetes mellitus. The distinct advantages of MSPCs over the conventional modalities and other pharmaceuticals may therefore lead to a broader range of applications of MSPCs in the field of regenerative medicine in the future.

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