

REVIEW

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Progress and emerging techniques for biomaterial-based derivation of mesenchymal stem cells (MSCs) from pluripotent stem cells (PSCs)

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Abstract

The use of mesenchymal stem cells (MSCs) for clinical purposes has skyrocketed in the past decade. Their multilineage differentiation potentials and immunomodulatory properties have facilitated the discovery of therapies for various illnesses. MSCs can be isolated from infant and adult tissue sources, which means they are easily available. However, this raises concerns because of the heterogeneity among the various MSC sources, which limits their effective use. Variabilities arise from donor- and tissue-specific differences, such as age, sex, and tissue source. Moreover, adult-sourced MSCs have limited proliferation potentials, which hinders their long-term therapeutic efficacy. These limitations of adult MSCs have prompted researchers to develop a new method for generating MSCs. Pluripotent stem cells (PSCs), such as embryonic stem cells and induced PSCs (iPSCs), can differentiate into various types of cells. Herein, a thorough review of the characteristics, functions, and clinical importance of MSCs is presented. The existing sources of MSCs, including adult- and infant-based sources, are compared. The most recent techniques for deriving MSCs from iPSCs, with a focus on biomaterial-assisted methods in both two- and three-dimensional culture systems, are listed and elaborated. Finally, several opportunities to develop improved methods for efficiently producing MSCs with the aim of advancing their various clinical applications are described.

Keywords Mesenchymal stem cell (MSC), Induced pluripotent stem cell (iPSC), iPSC-derived MSC, MSC derivation, Biomaterial

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Introduction

The use of stem cells for cell therapy has recently increased. This is especially true for adult stem cells, which are used to treat various injuries. Mesenchymal stem cells (MSCs) are a type of adult stem cells that have shown promising potential in clinical trials for treating various diseases [1, 2]. The therapeutic properties and large-scale use of MSCs have been researched extensively, and different methods have been developed for their isolation and production. MSCs are used owing to their self-renewal capabilities, multipotency, paracrine effects, immunoregulatory, and other cell-supportive properties [3, 4]. They regulate their own survival and impact the growth of neighboring cells or cells in the transplanted site. Their differential potential has been studied comprehensively and explored for the production of osteoblasts, adipocytes, chondrocytes, neurons, and myocytes [5]. These fibroblast-plastic-adherent cells are identified and isolated based on the presence of cluster of differentiation (CD) markers, including CD73, CD90, CD105, and stromal precursor antigen-1 (Stro-1) markers, and the absence of CD34, CD45, CD19, CD11, and human leukocyte antigen (HLA)-DR markers. This surface marker characterization was established by the International Society of Cellular Therapy (ISCT) to separate MSCs from heterogeneous populations of mononuclear adherent cells [6]. In addition to their multipotency, MSC sources are present throughout the body with little variation in their differentiation potential and surface markers across locations, and all of them can be used for cell therapy [5].

Generally, there are two mechanisms underlying the action of MSCs in treating diseased or damaged tissues or cells. The first is attributed to their multilineage differentiation capacity. They home to and engraft within injured sites and then differentiate into end-stage functional cells, thereby replacing the injured cells at those sites. Many articular cartilage injuries, for instance, injured knees, are treated by introducing MSCs into the damaged tissue, which then differentiate into articular chondrocytes to replace the damaged cartilage [7]. The second is the paracrine mechanism of MSCs. It has been well established that MSCs release various cytokines, such as angiogenic and neurogenic factors and immunomodulatory cytokines [8–10]. These factors promote the repair and regeneration of damaged tissue. Although MSCs have been widely used for clinical cell therapy, they have few limitations. For instance, MSCs have a limited self-renewal capacity when cultured in vitro. In addition, studies have demonstrated that they tend to lose their regenerative properties when cultured for long periods [11]. Furthermore, their source must be managed properly and subjected to quality control to avoid batch-to-batch variations. With the current developments in

induced pluripotent stem cell (iPSC) technology, iPSC-derived MSCs can eliminate the drawbacks associated with the MSCs derived from the existing sources.

This review examines the molecular morphology and medical applications of MSCs in treating various diseases and injuries. Here, the existing sources of adult MSCs are compared, with a focus on their isolation methods, proliferation rate differences, and multilineage differentiation potentials. Moreover, limitations in the clinical use of adult MSCs and approaches to overcome these drawbacks by generating MSCs from iPSCs are discussed. Finally, the existing methods for the differentiation of iPSCs into clinically relevant MSCs are identified and described. Moreover, recent advancements in two-dimensional (2D) and three-dimensional (3D) biomaterial-based directed differentiation of iPSCs into MSCs and the effects of various inducing growth factors are listed and compared.

MSCs: characteristics and potential promise for cell and tissue engineering

With changing lifestyles, health issues are increasing, and new drugs are being developed to treat these issues. However, therapeutic drugs often have serious side effects and low success rates. Cell therapy is an alternative and promising treatment approach. Cell therapy involves the use of cells instead of chemicals, and it often yields similar results in vitro and in vivo. For instance, when MSCs are used therapeutically at a bone injury site, they can expand, differentiate into osteoblasts, and release essential growth factors and paracrine molecules that support osteoblast proliferation [12]. Human embryonic stem cells (hESCs), iPSCs, and MSCs are among the most suitable sources of stem cells for use in cell therapy. It is important to note that the use of ESC is still debatable due to the possibility of teratoma formation and the ethical concerns regarding their isolation (destruction of an embryo) and thus are less preferred compared with iPSCs [13]. By contrast, MSCs are proven safe and have been used in several clinical trials [14, 15]. They are multipotent cells that can self-regenerate and can be grown in vitro. MSCs possess fibroblast-like morphology with plastic-adherent ability, which allows them to grow in tissue culture plates without requiring additional substrates.

Cellular and molecular properties of MSCs

MSC-like cells were first discovered by Friedenstein in 1970 in guinea-pig bone marrow (BM) [16]. However multiple cell types in the BM led to controversy in selecting a specific name for these cells. Later, the ISCT termed these cells multipotent mesenchymal *stromal* cells based on specific properties such as having a fibroblast morphology, and plastic-adherence capability [17].

Later, many surface markers specific to these cells were found; the CD on their surface allowed for the identification of MSCs from other groups of plastic-adherent cells. The presence of CD73, CD90, CD105, and Stro-1 and the absence of CD45, CD34, CD14, CD19, and HLA class II identified the presence of MSCs in cell cultures [18–22]. These characteristic markers are used for their isolation as well.

Slight differences in the expression of surface markers may occur based on the origin of MSCs. Depending on the source of MSC (adipose tissue, bone marrow, umbilical cord), some MSC populations have different cell surface markers, which may contribute to and be reflected in their differentiation potentials [5, 23–25]. These differences allow the selection of a particular type of MSCs for a specific therapeutic purpose.

MSCs are multipotent cells that can differentiate into different types of cells. Generally, they are recognized for their potential to differentiate into osteoblasts, chondrocytes, and adipocytes. However, they not only differentiate into mesoderm-type cells but also into other lineages, such as cardiomyocytes, neurons, germ cells, and hepatocytes, and this is achieved by altering various signaling pathways during the development of MSCs to induce specific differentiations [26–28]. Treating different growth factors can alter the differentiation potential of MSCs [26, 28, 29].

Another important property of MSCs is their ability to dodge the immune system and improve transplantation efficiency. The major histocompatibility complexes MHC-I and MHC-II are proteins that play a major role in the recognition of allogenic cells by the host immune system. Low expression of these molecules in MSCs enables them to be not recognized by the host's immune system [8, 30, 31]. In addition, the immunomodulation capability of MSCs promotes angiogenesis, inhibits apoptosis and inflammation, and modulates the extracellular matrix (ECM) through paracrine factors [32]. They are also involved in suppressing the proliferation of T cells and the maturation of dendritic cells (DCs) and monocytes [33]. Overall, MSCs can control the immune regulatory system of the body by promoting or suppressing proteins that have important functions in the immune system.

The paracrine effect of MSCs additionally plays a major role in injury treatment and cell growth. MSCs release around 43 angiogenic factors, among which 11 factors are involved in bone regeneration; 2 in neuroprotection; and the other 30 in vascular repair, peripheral artery disease, and myocardial infarction treatment [9, 10, 34]. Moreover, immunomodulatory effects are managed by paracrine factors. making release of cytokines and growth factors has been observed to inhibit immune cell proliferation [35]. Overall, these results demonstrate that the properties of MSCs can significantly affect healing

and regeneration. A diagram depicting all these characteristics is presented in Fig. 1.

Clinical applications of MSCs

Owing to the low efficacy and efficiency of drugs available for treating several diseases, a shift has occurred toward cell therapy, and recognition of the therapeutic potential of MSCs is perhaps the most exciting advance in cell therapy [36–38]. Recently, MSCs have been profusely used in cell replacement strategies to treat cartilage injuries caused by chronic load, autoimmune diseases such as arthritis, and physical trauma. For instance, autologous transplantation of BM-derived MSCs embedded in a 2-mm-thick collagen gel onto a patient's patella led to improvement in the patient's movement and promoted complete regeneration of the cartilage tissue [39–41]. MSCs derived from other sources have yielded similar results, thereby proving the benefits of MSCs in treating osteoarthritis and other cartilage-associated defects [7, 40–47].

Furthermore, MSCs have been used to treat various bone defects caused by bone loss, fragility, and fracture. With technological advancements, various biomaterials have been used in combination with MSCs to achieve improved results. MSCs were successfully combined with a bovine bone mineral to reconstruct the atrophic maxilla [48]; this approach facilitated bone formation in the elevated region owing to the presence of MSCs in the biomineral. Similarly, MSCs combined with bio-ceramics have been used to treat long bone non-unions [49].

Other clinical applications of MSCs include treatment of spinal cord injuries, graft-versus-host diseases (GVHDs), and various cardiovascular diseases [50–54]. Moreover, the injection of autologous BMMSCs at injury sites led to improvements in motor power, daily motor tasks, and recovery of injured areas. These improvements, however, were subjective and patient-dependent [50, 51].

Although the use of MSCs for cell therapy has paved ways in treating various diseases, there are still some limitations. First, adult MSC have limited proliferation capacity. Thus, a constant supply of adult MSCs is needed to sustain treatment. Although this can be solved by obtaining MSCs from several donors, cells obtained from various donors and tissues have differences. In general, MSC condition depend on the donor's sex, age, race, and existing pre-medical conditions (Fig. 2) [55, 56]. A detailed explanation of the limitations of MSCs is discussed in the following sections.

Current sources of MSCs and their limitations

It has been well established that MSCs derived from various tissues and donor sources have different proliferation and differentiation potentials. Therefore, it is imperative

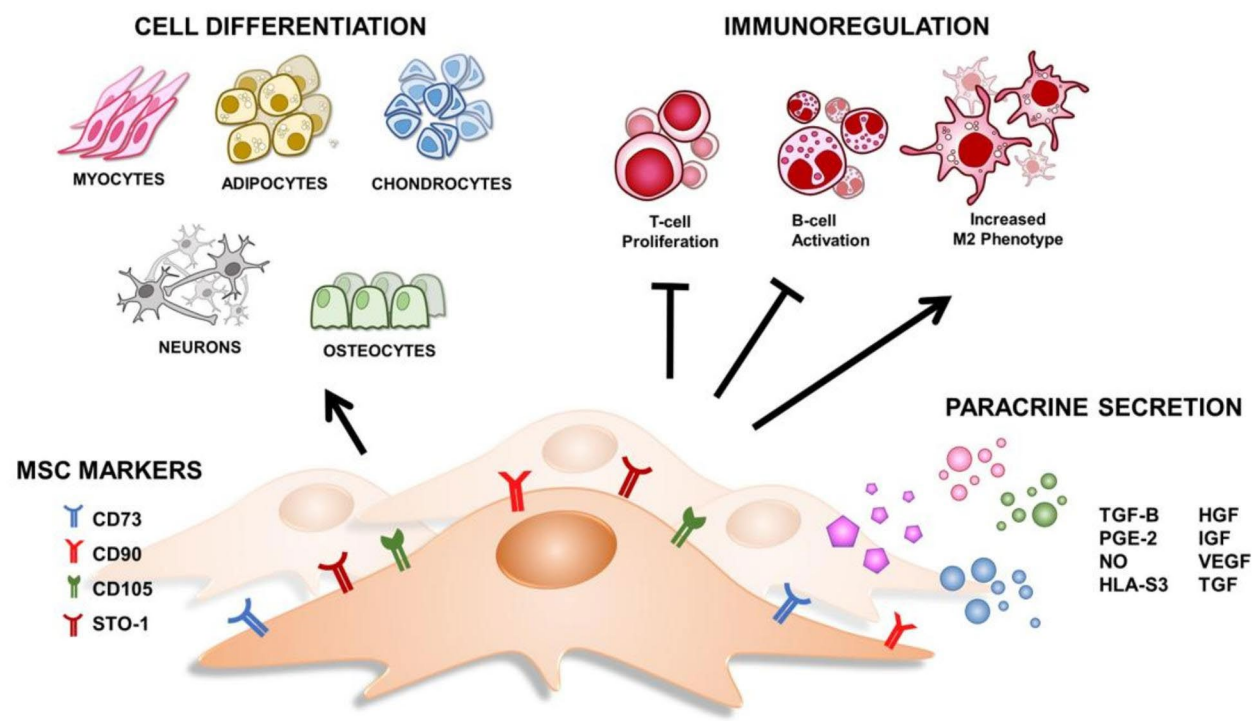


Fig. 1 Cellular and molecular properties of MSCs. MSCs display multipotency and different CD markers. They are great candidates for immune-regulation by means of T-cell and B-cell inhibition, as well as M2 phenotype encouragement. Paracrine secretion of different growth-regulating and inhibitory factors provides them distinct therapeutic properties to support other cells

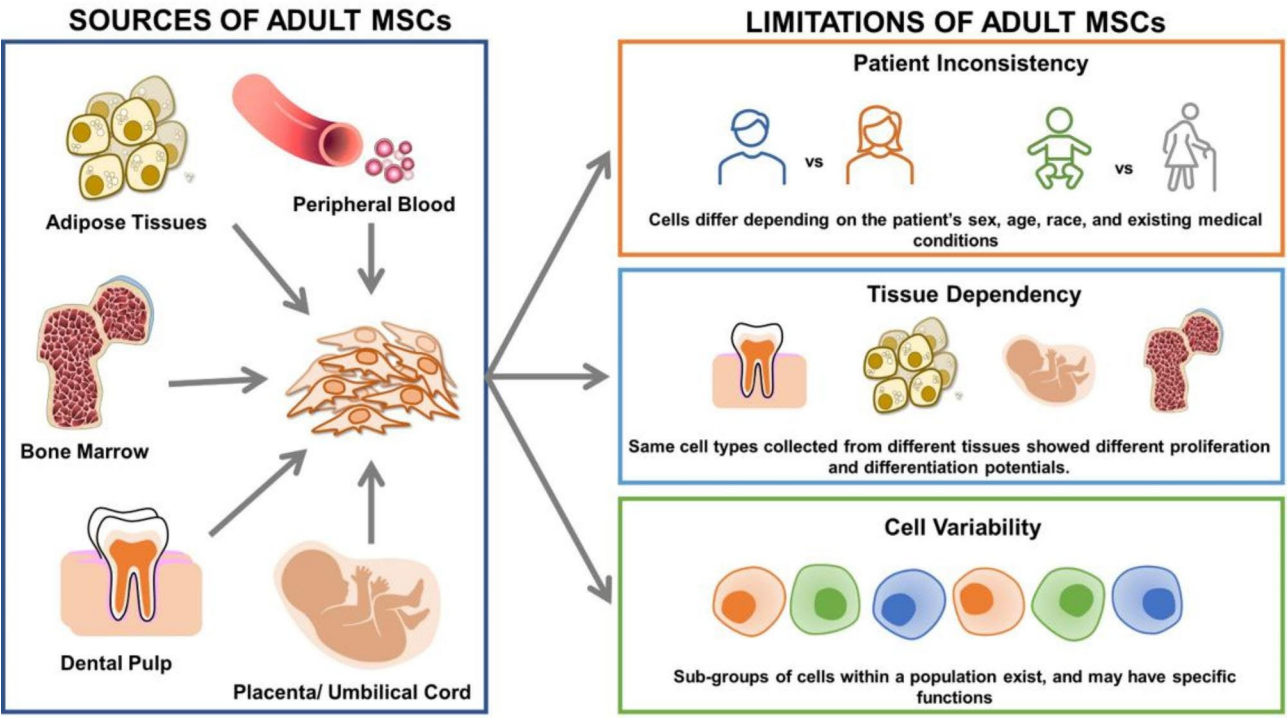


Fig. 2 Sources and heterogeneities of MSCs. Heterogeneity in MSCs can be classified into different levels: donor-to-donor inconsistencies, tissue-dependent variations, and the presence of sub-populations. Due to these variations, the therapeutic potentials of MSC are hindered, which encouraged scientists to shift their focus towards the development of a new source of MSCs.

to know the source of MSCs to establish whether they are fit for purpose and ensure a successful outcome. These sources can be categorized into two types based on the age of the donor: adult and infant sources. Adult tissue sources include BM, AD, dental pulp, peripheral blood, synovium and synovial fluid (SF), endometrium, skin, and muscle. Infant tissue sources include amniotic fluid, the placenta, umbilical cord blood (UCB), and Wharton's jelly (WJ) [5, 57].

In general, adult-sourced MSC are easily accessible but with lower proliferation capacity. On the other hand, infant-sourced MSCs have higher proliferation but are not always available [14]. Specific differences and limitations are discussed in the subsequent sections.

Adult tissue sources

The major sources of adult MSC in the human body are bone marrow and adipose. BM was considered the only multipotent MSC source after its discovery in 1970 [58]. Isolation of MSCs from BM is rather invasive and painful, but cells isolated from BM can differentiate into adipocytes, chondrocytes, osteocytes, cardiomyocytes, neurons, and many other types of cells [5, 59]. The immunoregulatory effects of these cells are weaker than those of umbilical cord-derived MSCs (UCMSCs), but their immunomodulatory properties are adequate, and they promote angiogenesis, myogenesis, and growth and development of other cells, which make them a great candidate for cell therapeutics [8, 60, 61].

Another source of MSCs is AD tissue, which is easily accessible and abundant throughout the body. Adipose-derived MSCs (ADMSCs) are isolated from the subcutaneous AD tissue harvested by liposuction or as large pieces of tissue [62]. They exhibit high plasticity, which leads to differentiation into various cell types, such as adipocytes, chondrocytes, osteoblasts, cardiomyocytes, neuronal cells, hepatocytes, and, more recently, germ cells; moreover, they have immunosuppressive effects and release more angiogenic regulators [5, 63–65].

Dental pulp-derived MSCs (DPMSCs) are isolated from the dental pulp tissue of the third molar teeth, deciduous teeth, or any extracted teeth [66, 67]. They are easier to isolate, have a high proliferative rate, and provide adequate amounts of MSCs for therapeutic use, despite the small area of teeth. Nonetheless, their differentiation potency is limited to neural ectoderms, myoblasts, osteoblasts, chondrocytes, adipocytes, and odontoblasts, compared to that of bone marrow-derived mesenchymal stem cells (BMMSCs), which have vast plasticity [67]. Other sources of MSCs include synovium, SF, and synovial membrane [68–70].

Infant tissue sources

Similar to adult tissue sources, MSCs can be isolated from various infant tissues. These sources of MSCs are available starting from embryo development until the infant's birth, and they include tissues from the embryo and the mother [25].

The umbilical cord (UC) is a tissue composed of the umbilical arteries (UCAs) and umbilical veins (UCVs) and is surrounded by a gelatinous extracellular membrane called WJ [71]. UCs are considered medical waste and are, therefore, an easily and noninvasively available source of MSCs. MSCs isolated from UC arteries and veins are often called UCMSCs while the ones isolated from the WJ are called WJMSCs. It is important to note, however, that these terms are used interchangeably in most publications. These UCMSCs/WJMSCs have a high proliferative rate, short doubling time, are abundant, and exhibit excellent immunosuppressive properties, and were found to prefer chondrogenic differentiation [31, 72–78].

Umbilical cord blood (UCB) is another source and termed as UCBMSC that is abundantly available and easily retrieved [79, 80]. These MSCs have high doubling number, can be cultured for higher passages and can efficiently differentiate into osteocytes and chondrocytes compared to BMMSC and ADMSC. In some studies, it is suggested that they outperform WJ-MSCs by releasing anti-fibrotic factor HGF and genes associated with ECM remodeling, which are helpful in scarless wound healing [79–81]. Likewise, placenta and amniotic fluid demonstrated MSC like properties with good immunogenic properties and high proliferative rate [82–84].

Limitations and heterogeneity of existing MSC sources

Although most types of MSC have same cellular morphology and properties, these cells are also found to have differences in molecular phenotype and thus functions. The heterogeneity among various MSC populations can be subdivided into three categories: Donor-to-donor inconsistencies, tissue-dependent variations, and existence of cell sub-populations. The heterogeneity of MSCs is depicted in Figs. 2 and 3.

The first level of heterogeneity in MSC is the donor-to-donor variations. The age, sex/gender, and the presence of pre-existing medical conditions of the donors are found to affect MSC proliferation, differentiation, and therapeutic potentials. For instance, BMMSCs derived from a female donor showed higher therapeutic efficacy compared to BMMSC from a male donor [56]. Similarly, MSCs isolated from young donors have higher proliferation, increased anti-inflammatory cytokine secretion, and enhanced immunoregulation compared to MSCs from an old donor [85]. Finally, the donor's pre-existing

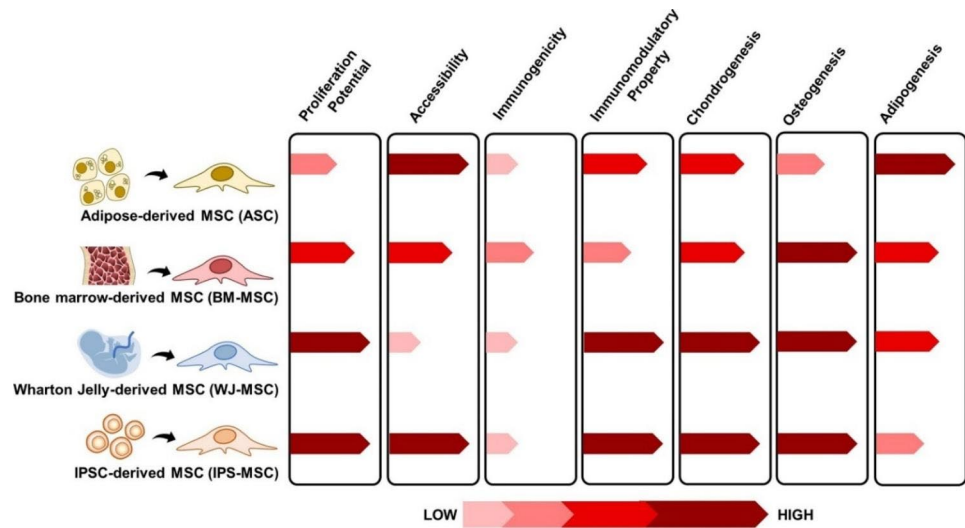


Fig. 3 Comparative analyses between several types of MSCs. MSC have multiple sources of origin such as bone marrow, adipose tissue, umbilical cord, dental pulp, and peripheral blood. Each source has varying level of accessibility and isolation method. Moreover, depending on the source of MSC, differences in proliferation capacity, differentiation potentials, immunogenicity, and immunoregulation are observed. Comparison was based from the most current studies on MSC.

Table 1 Different existing sub-populations of MSC. Various sub-populations exist within a particular type of MSCs. Based on the presence of cell surface markers, some sub-population may have different properties, and thus may be used for a specific function

MSC subpopulation	Source	Differentiation capacity and Enhanced Properties	References
CD105+	AD and UC	Enhanced angiogenesis, chondrogenesis, and increased proliferation	(97)
CD105-	AD and UC	Enhanced osteogenesis, higher immunosuppressive ability	(91,98)
CD200+	BM and UC	Enhanced osteogenesis and immunoregulation	(92)
STRO-1+	BM, DP, SM	Arteriogenesis, cardiac recovery and homing ability	(88)
CD271+	AD, BM and synovium	High CFU, enhanced HSC engraftment, superior chondral repair, high proliferation	(90,91)

medical conditions and genetic variations are other types of limitations that affect MSC properties [11, 86].

The next level of heterogeneity is the tissue-specific differences of various MSCs. Depending on the tissue source, isolation procedure, accessibility, and differentiation potentials may vary. BMMSCs have been used in clinical trials for osteogenic and chondrogenic injuries, as well as cardiovascular and neurogenic diseases, among others. BMMSCs have great potential, but their retrieval procedure causes pain, potentially life-threatening infection, and donor site morbidity in patients [87]. In addition, the quantity of harvested cells is limited. MSC collection from the BM isolates only approximately 0.001–0.01% of the total MSCs, which need to be expanded in vitro to a higher passage number. Consequently, the isolated cells lose their potency and genetic stability [65, 88, 89]. In terms of differentiation potentials, BMMSCs have better chondrogenic and osteogenic potentials than adipogenesis.

On the other hand, adipose tissues (AD) provide ten-fold more MSCs than bone marrow. These tissues are more accessible, and the tissue collection is less invasive compared with the bone marrow [24]. Having an adipose

origin, ADMSCs prefer adipogenesis but are also effective in capable of osteogenesis and chondrogenesis. In contrast, UCMSCs involves a more noninvasive isolation method and provide an abundant supply of stem cells, but they are not always available timeously because they can be obtained only when an infant is born, which limits their supply [75]. Compared with other sources, UCMSCs can efficiently undergo chondrogenesis, osteogenesis, and adipogenesis.

The final level of heterogeneity involves the presence of several sub-populations of MSCs.

For instance, the CD105[−] ADMSCs exhibit stronger osteogenic potential than the CD105⁺ cells [90–93]. In a parallel study, CD271⁺ BMMSCs are superior in repairing articular cartilage defects [94, 95]. On the other hand, CD200⁺ BMMSCs have shown better osteogenic and immunoregulatory effects than CD200[−] MSCs [96]. A list of various sub-populations of MSCs are listed in Table 1.

These underlying heterogeneities of MSC populations hinders its therapeutic potentials. Although adult- and infant-sourced MSCs have been widely used in cell therapies, they are insufficient as there is still a need for a constant supply of healthy, highly proliferative, and

homogenous population of MSCs for medical purposes. Thus, there is a high demand to differentiate PSCs into functional MSCs. For instance, in recent years, MSCs have been derived from iPSCs, and they are called induced mesenchymal stem cells (iMSCs). iMSCs have reformed the MSCs used in therapeutics because they overcome the limitations of conventional MSC sources. Based on the most recent studies in MSC, a comparison between BMMSC, ASC, UCMSC, and PSC-derived MSC is shown in Fig. 3.

Biochemical and physiochemical factors affecting MSC fate

To better understand MSC development, it is important to understand the micro-environment or niche in which these cells reside in-vivo. Several factors associated with these niches, such as biochemical and physiochemical cues, regulate molecular processes such as cell proliferation, differentiation, and survival. Understanding these microenvironments in-vivo allows scientists to mimic cell development in-vitro and maximize the use of cells, in this case MSCs, for therapeutic purposes. Because MSCs have multiple tissue sources, we focused on discussing the bone marrow microenvironment where BMMSCs are located. The BM microenvironment is a dynamic region composed of several cell types, growth factors, and cytokines. It is a rich area that supports the production and development of all blood cells in the body. In this section, we chose to discuss BMMSCs as these cells are one of the longest and most utilized cell sources for stem cell therapy. In comparison with other types of MSCs, BMMSCs have shown better multi-lineage potential than ADMSCs, and are more accessible compared with UCMSCs. Finally, the biochemical and physiochemical cues provided by the BM for BMMSCs proliferation, migration, differentiation, and paracrine secretion have also been well established.

Biochemical factors affecting MSC development

In bone marrow, MSCs reside and interact with various cells such as osteoblasts, osteoclasts, immune cells, pericytes, and hematopoietic cells. These cells secrete various factors and signaling molecules that affect MSC proliferation, fate decision, paracrine secretion, and differentiation. A complex network of signaling pathways is involved in MSC development, but only a few MSC signaling pathways have been explored thus far. These signaling pathways are strongly influenced by several growth factors, hormones, and cytokines [99, 100]. Well-studied key regulatory factors include growth factors such as TGF β 1, IGF1, bFGF, and VEGF and cytokines such as IL-6, IL-1 β , IFN- γ , and TNF- α . In addition to these, oxygen, calcium, and the other hormones that are

generally released in diseased conditions play an important role in MSC development [101, 102].

In the BM, numerous studies suggest that MSCs are in quiescence (G0) state in the perivascular spaces. When the body perceives any local or distant injury or tissue damage, they become activated, are induced to proliferate, and are signaled to migrate to injury sites to promote healing and tissue repair [103]. Depending on the location in which MSCs are needed, the MSCs are then induced to differentiate into a specific cell type, i.e. osteoblasts for bone repair or chondrocytes for cartilage repair. The ability of the MSCs to self-proliferate or also called “stemness” is regulated by cytokines and growth factors such as IL-6 the TGF β proteins, BMPs, EGF, PDGF, and others [104]. Activated and proliferated cells then migrate and home to the damaged tissue via the upregulation of Stro-1 surface proteins [105]. Here they differentiate in response to the upregulated cytokines or growth factors in the injury site. In bone fractures, for instance, macrophages in the injury site secrete IL-1, IL-6, and tumor necrosis factor-alpha (TNF- α) that recruits MSC to start the process of healing and osteoblast differentiation [106]. Other growth factors of MSCs proliferation and differentiation are discussed below.

One of the most abundant growth factors present in bone matrix is IGF-1, and has been determined to be involved in the proliferation, self-renewal, and in some parts, osteogenic differentiation of MSCs [107–109]. IGF-1 is primarily involved in proliferation, but during the process of MSC differentiation, it additionally helps with the mineralization of cells through the activation of the mTOR pathway, indicating its possible dual role in MSC regulation [107, 110]. In a parallel study, the presence of IGF-1 promoted the enhanced proliferation of MSCs under hypoxic conditions [111].

Another abundantly available growth factor (GF) in the BM microenvironment is the TGF β family of proteins composed of TGF β 1, TGF β 2, and TGF β 3, and all BMPs. It is well established that the TGF β family regulates MSC development. For instance, TGF β 1 binds to MSCs during bone remodeling, induces the migration of MSCs to the target location, and promotes the chondrogenic and osteogenic differentiations of MSCs [99, 112, 113]. Another protein belonging to the same family is BMP4, which is primarily involved in adipocyte generation [114]. The TGF family has additionally been found to regulate MSC proliferation through crosstalk with the Wnt signaling pathways [115]. A study demonstrated that TGF β 1 supports Wnt signaling through the activation of Smad3, which induces enhanced nuclear translocation of β -catenin, and thus, increased proliferation. Interestingly, this crosstalk reduces the osteogenic differentiation of MSC and is, therefore, important for maintaining the self-renewal capacity of MSCs [116]. Similar crosstalk

was observed between TGF β 1 and FGF2, which promotes MSC proliferation, whereas HGF and IGF-1 promote osteogenesis [117].

Other chemical cues present in the bone microenvironment include several cytokines and oxygen. Cytokines such as TNF α , IL-1 β , and IL-6 were found to be involved in the differentiation of MSCs into osteoblasts [99]. Oxygen, by contrast, has been found to regulate both the proliferation and differentiation of MSCs. A lower oxygen concentration supports MSC proliferation, while a higher oxygen concentration induces osteogenesis and angiogenesis [111, 118].

Physiochemical factors affecting MSC development

To develop a suitable biomaterial-based platform for the differentiation of iPSCs into MSCs *in vitro*, it is important to identify the physiochemical factors that regulate MSC development *in-vivo*. Herein, factors such as ECM composition, microenvironment stiffness, and topography are discussed. The bone is a spongy microenvironment comprising several minerals (hydroxyapatite, calcium), proteins (ECM proteins, Type I collagen, laminin), lipids, and water [119]. The concentration and arrangement of these molecules ultimately affect bone fragility and help maintain angiogenesis, oxygen regulation, and overall growth of MSCs [120–122].

Collagen is one the most abundant ECM proteins in BM. The presence of collagen not only provides tensile strength but also allows for the mineralization of calcium and other ions and eventually creates a special microenvironment for cell growth and survival, including MSCs. Additionally, collagen allows for enhanced cell adherence and proliferation of MSCs by reducing their doubling time [123]. Other ECM proteins such as fibronectin and laminin, too, regulate the development of MSCs by interacting with the various integrins expressed by MSCs [124, 125]. Aside from ECM proteins, several minerals have been found to determine the fate of MSCs in the BM niche. For instance, calcium ions enhance bone regeneration by promoting MSC migration and inducing osteoblast generation [126]. Such regulated calcium release was achieved *in vitro* on a hydroxyapatite scaffold to induce osteoblast differentiation of MSCs [127].

BM is composed of the endosteal niche, which is heavily populated with various bone-forming cells, and the vascular niche, which is populated with endothelial cells, adipocytes, and stromal cells [128, 129]. These niches differ in terms of cell composition, stiffness, oxygen concentration, and for this reason, they have varying effects on cell development in BM. MSCs can be localized in both niches, and therefore, it is imperative to note that the properties of these niches affect the general fate of MSCs [130]. The endosteal niche is relatively stiffer (35–40 kPa) than the vascular niche (3 kPa). *In vitro*

studies have attempted to determine the effect of ECM stiffness on MSCs by mimicking its *in-vivo* condition. It has been demonstrated that the stiffer fibronectin-coated polyacrylamide hydrogel (13–68 kPa) supports BMMSC adhesion and spreading [131]. Moreover, chondrogenic and osteogenic differentiation has been found to be greatly enhanced when BMMSCs are seeded in 4-methyl acrylate polymer, which has a stiffness of 10 MPa [132]. This can be attributed to the fact that *in vivo*, bone formation usually occurs in the stiffer endosteal niche, where MSCs are recruited and programmed to differentiate into bone-forming cells. Other important properties of this niche that can affect MSC fate include porosity and topography of the ECM. Although only a few studies have been conducted to determine these factors *in-vivo*, several attempts have been made to determine them *in vitro*. In Vissers et al.'s study, a scaffold with a pore size of 500–850 μ m, exhibited the strongest osteogenic response [133]. In a similar study, a scaffold with cubic pores greatly increased the gene expression of MSCs undergoing chondrogenesis and adipogenesis compared to a scaffold with cylindrical pores [134]. In terms of topography, it is known that cells can detect surface patterns of matter from 10 nm to 100 μ m and control their ability to differentiate and proliferate. In one study, a 15- μ m ridge increased the efficiency of adipogenic differentiation, while a 2- μ m ridge increased the efficiency of osteogenic differentiation [135]. In another study, the MSCs grown on micro-grooved surfaces exhibited higher expressions of pluripotency-related markers and two to threefold faster proliferation capabilities compared to those of the MSCs grown in a conventional culture flask [136].

Existing strategies for differentiating PSCs into MSCs

Because the sources of MSCs are limited and incapable of retaining their characteristics for long periods in *in-vitro* environments, a stable source of MSCs is required. Pluripotent stem cells (PSCs) have the potential to overcome these limitations and serve as a superior source of MSCs than the existing sources. This conversion is directed through a particular route. Conventionally, PSCs are first converted into mesodermal cells and then differentiated into MSC-like cells. A few researchers have directly converted PSCs into MSCs. In one study, MSC-like properties were induced in somatic cells by transducing human adult blood CD34⁺ with lentivirus OCT4 under MSC-inducing conditions [137]. The different routes of and recently developed methods for producing MSCs from iPSCs are described below and summarized in Table 2, while the advantages and disadvantages of each technique is summarized in Table 3.

Table 2 Existing techniques for generation of MSCs from PSCs. Various methods such as growth factor induction, embryonic body formation, gene modification, and biomaterials have been utilized to differentiate various PSCs into MSCs.

Type of Technique	Method	Cells used	MSC properties	Publication
Growth Factors	BMP4	hESCs	EMT gene upregulation	(158)
	BMP4 with activin inhibitor (A83-01) in MesenCult™ media	hESCs	3–4 weeks for differentiation; CD73 ⁺ , CD90 ⁺ , and CD105 ⁺ cells with trilineage properties	(135)
	ESCs were cultured with SB431542	hiPSCs	MSCs were generated rapidly in 10 days, and they exhibited good trilineage potential and efficient passaging capability	(140)
	IκB kinase/NF-κB signaling inhibitor and p65 inhibitor were used with MSC culture medium	hiPSCs	MSCs with high osteogenic and chondrogenic properties	(155)
Embryoid body	ESCs were cultured in suspension culture and outgrowths were isolated	hESCs	10-day differentiation; CD73 ⁺ , CD90 ⁺ , and CD105 ⁺ cells with chondrogenic properties	(147)
	ESCs were cultured in suspension culture. The EBs formed were plated in 0.1% gelatin low-glucose media, and outgrowths were isolated	hESCs	MSCs markers were observed, and the expressions of the CD73 and CD105 markers increased with the passage number	(148)
	Cells were plated in low-attachment plates, and the EBs formed were replated in 0.1 gelatin with MSC media	iPSCs	After 35 days, MSC-like cells were generated with low CD105 expression, immunomodulatory properties, and adipogenic potential	(149)
	EBs were formed in non-tissue culture plates with knockout serum replacement medium, transferred to the MSC medium, and treated with SB431542	iPSCs and hESCs	MSCs were generated in 10 days with SB431542 treatment; the CD105 and adipogenic potential were low. The other markers were normal, osteogenic and chondrocyte potential were normal, and proliferation rate was higher	(139)
	EBs were formed with BMP4, bFGF, and VEGF on a low-attachment surface. Mesangioblast were formed, which were then cultured in serum-free methylcellulose medium over Matrigel®	hESC	The output was higher than that of BMMSCs with higher doubling rate and smaller size; the Stro-1 marker was lacking	(160)
	OP9 stromal cells were co-cultured with hESC; CD73 ⁺ cells were isolated with FACS and cultured	hESC	Induction time was longer at 40 days. Other than CD90, all markers were present, and the trilineage potential was good	(150)
Other techniques	Repeated passage of ESCs on gelatin-coated plates	hESC	Passage pressure generated MP, which exhibited homogeneous population, tumor-free nature, and good tri-lineage potential	(155,161)
	Lentivirus-induced HOX genes in miPSCs	miPSCs	HOX gene was overexpressed, and vascular wall MSCs were generated	(152)
	DOX-induced MSX2 overexpression in hiPSCs in MSCs medium	hiPSCs	Overexpression of MSX2 generated immature MSCs, but with small molecules, mature MSCs were generated, and their trilineage potential was similar to that of BMMSCs	(138)
	EZH2 inhibitor was added to hESC medium, and cells were sorted	hESC	MSCs were generated within 7 days; no CD105 markers were used, and the generated MSCs had low adipogenic and chondrogenic potential but good osteogenic potential	(153)

Growth factor and small molecule-induced differentiation
The proliferation and differentiation of cells depend on their signaling pathways. These pathways involve intrinsic or extrinsic signaling molecules. Cells do not experience fate change without utilizing these signaling molecules, and same is the case for MSCs. A complex network of signaling pathways is involved in MSC proliferation, differentiation, and generation. However, limited MSC signaling pathways have been explored thus far. Recently, MSCs have been generated from PSCs by manipulating environmental and signaling cues during PSC culture.

BMP4 has been used to obtain MSCs from the mesodermal and trophoblast lineages by varying FGF2 incorporation [138–140]. Similarly, iPSC to MSC

differentiation can be induced through the ectodermal lineage route. Zhang et al. identified neural crest cells (NCCs) as a fast and efficient route for MSC generation through MSX2 overexpression [141, 142]. MSX2 has been found to be affected by Smad1/5/8, which is a main downstream molecule in the BMP4 pathway. Therefore, BMP4 can regulate MSX2 expression to differentiate iPSCs into NCCs or mesendodermal cells. Apart from growth factors, the small molecule SB431542 has been used to derive MSCs from iPSCs [143–145]. This molecule inhibits the phosphorylation of Smad2/3, which, in turn, inhibits TGF-β1 signaling in the ESCs that generate mesodermal progenitors (MP) [144].

Table 3 Advantages and disadvantages of various PSC to MSC differentiation techniques. Some differentiation techniques are superior than others in terms of length (time), economy (cost), the level of difficulty, and the yield (number of differentiated cells) of the technique

Techniques		Advantages	Disadvantages
Growth Factors		Easy and faster treatment, Multiple growth factor options available Efficient in 2D differentiation. Production of more homogeneous cell population	This method involves 2D differentiation thus does not mimic human in-vivo condition. Longer differentiation time that requires numerous sub-cultures. Requires enormous amount of growth factors
Embryoid Body		Mimics human physiological (in-vivo condition). Fast, more effective, and more efficient differentiation.	Possibility of necrotic zone formation. Production of heterogeneous cell population
Other techniques	OP9 monolayer cells	OP9 cells provide the necessary supplements, cytokines and growth factors for MSC differentiation. Results to production of cells with better trilineage differentiation.	Possibility of animal cells contamination. Longer differentiation time Additional sorting step is required
	Selective pressure	Homogeneous cell population. Animal free cells, Results to production of cells with better trilineage differentiation.	Longer time required, Negative effect of trypsin over cells, High batch-to-batch variations due to the complete dependency on cell passaging
	Genetic manipulations	Faster differentiation, Effective and efficient differentiation Results to production of cells with better trilineage differentiation.	Trained laboratory technicians required. Costly, Possibility of genetic mutations.
	Epigenetic inhibitor	Fast differentiation, good osteogenic potential	Results to production of cells with poor trilineage differentiation.

Wnt and basic fibroblast growth factor (bFGF) have been observed to affect MSC generation. A few researchers have used CHIR, a GSK3 β inhibitor, to induce MSC characteristics in iPSC-derived NCCs, while other researchers have used bFGF for similar purposes [138, 139, 142, 146]. Other growth factors, siRNA and different small molecules used for MSC generation are listed in Table 2.

EB formation

Embryoid body (EB) formation changes the effect of a 2D environment on cell growth. EBs are 3D aggregates of PSCs cultured in suspension media. When left in suspension media or on non-adherent plates, ESCs or iPSCs accumulate and form aggregates that have spheroidal structures (143–145). A few EB aggregate formation methods are based on the forced self-aggregation, hanging drop, and AggreWell™ methods [147, 150]. EBs are widely used to provide a 3D platform for generating cells of different lineages [148, 149].

Interestingly, EB formation can be used to generate MSCs from PSCs. MSCs are generally obtained as an outgrowth from EBs because they have a fibroblast-like morphology and extend themselves on flat surfaces to ensure their growth and survival. EBs were first generated by culturing small clumps of ESCs in suspension culture for 10 days and then transferring them to gelatin-coated plates that generate MSC-like cells with increased expressions of the CD73 and CD105 markers [151–153]. Some of these cells were then cultured in 10% human platelet lysate under MSC culture conditions. After 35

days, the growth of fibroblast-like cells was observed. Various other methods have been developed, as summarized in Table 2.

Other techniques

Apart from the above-mentioned techniques for MSC generation, there are other methods that do not fall into the categories discussed herein but are important to produce MSCs from PSCs. One such technique is the use of murine OP9 stromal cells for deriving MSCs. Barberi et al. cultured hESC over OP9 monolayer cells to induce mesenchymal differentiation [154]. In this direct co-culture system, the OP9 cells provide the necessary cytokines and growth factors to support the differentiation of hESC to MSC. After several days of co-culture, the cells were harvested and sorted for CD73+ surface marker. The CD73+ cells were then expanded in α MEM without mouse stromal cells.

Another method is the inclusion of a xeno-free environment. Karlsson et al. attempted to derive mesenchymal progenitor cells (MPCs) through the selective pressure technique. First, ESCs were cultured in high-glucose media containing bFGF for several days. The cells were then enzymatically dissociated into single cells and sub-cultured on a new gelatin-coated dish. This process of cell culture, cell dissociation, and passaging were repeated several times until homogeneous cell populations are achieved [155]. After several passages, these cells that no longer express any ESC surface marker are then characterized as MPCs.

Another method to generate MSCs involves directly transducing iPSCs with *Hox* genes that are upregulated in vascular wall MSCs (VWMSCs) [156]. Lentiviral transduction of these factors differentiates mouse iPSCs into VWMSCs [142].

Probably the most recent technology, epigenetics has also been used as a unique method for differentiating PSCs into MSCs. The enhancer of zeste homolog 2 (EZH2), an epigenetic regulator, was inhibited, and hESCs were differentiated into MSCs three times more efficiently than that in the case without such inhibition [157].

Biomaterials affecting MSC development

Chemical cues play an interesting role in the differentiation of PSCs into MSCs and have been used widely. Mostly, growth factors, small molecules, and biomolecules such as RNA and DNA are used for this purpose, and they exhibit highly effective differentiation abilities. In spite of its great successes, this line of research has several limitations. For instance, the lack of biomimetic properties, unstable efficiency of growth factors, and tissue maturation are the drawbacks of utilizing only chemical cues to differentiate PSCs into MSCs. In addition to chemical cues, proper growth or maturation of cells can only be achieved through the regulation of their physical cues by using materials that can act as an ECM for cells. Biomaterials, which are common nowadays and have been utilized in many recent studies, successfully provide these physical cues. By using biomaterials, cell-cell and cell-matrix interactions are regulated efficiently. These biomaterials can also be conjugated with different growth factors (GF) which in turn stabilizes these GF and regulate their release into media for cellular consumption. In addition, cell adhesion, differentiation, and maturation can also be regulated by biomaterials [162]. It is important to note that the use of biomaterials to regulate cellular functioning varies depending on their properties, nature, formulation, and interactions with cells.

Types of biomaterials

Natural biomaterials

Natural biomaterials are from natural sources, such as human, plant, and animal sources. Owing to their organic origin, natural biomaterials are biocompatible and biodegradable. In addition, they possess all the factors required for cell adhesion, proliferation, and differentiation, which aids cellular functioning [163]. Materials in this category include proteins, polysaccharides, proteoglycans, and glycosaminoglycans, and each of them has specific advantages and disadvantages in terms of cell function. These naturally derived biomaterials have been used to culture different types of cells and develop

advanced medical products with increased cell implantation efficiencies [151].

Gelatin Gelatin is one of the most widely used conventional ECM proteins for preparing biomaterials. It is normally obtained from the irreversible denaturation of collagen protein, and both materials exhibit similar molecular properties and functions in cell culture and tissue engineering. Gelatin has been demonstrated to be effective in the isolation and mass production of early-stage BMMSC [164]. Gelatin contains the integrin binding moiety: arginine–glycine–aspartic acid (RGD) sequence. The binding of the integrins present on the surface of BMMSCs to the RGD peptide of gelatin promotes better attachment of cells to the substrate. The enhanced attachment in turn increased proliferation of cells, and thus mass expansion of BMMSC. [165, 166].

Collagen Collagen, the most common protein available in the human ECM, is a biomaterial that can promote the differentiation of MSCs into the osteogenic lineage [123]. Expansion on a collagen type I-coated plate is adequate to induce high levels of osteogenic differentiation. Pericellular collagen I facilitate MSC adhesion and differentiation into chondrocytes [167]. It is important to note that compared with gelatin, the highly organized structure of collagen is still superior in promoting cell adhesion and proliferation at any seeding density [168]. This indicates that the full version of the ECM protein is better in activating cellular processes. However, gelatin is way cheaper and readily available. Moreover, gelatin is highly soluble and modifiable which are essential characteristics for biomaterials. Because of these reasons, gelatin is utilized more, especially in routinary cell culture experiments.

Other Natural Biomaterials Recently, silk fibroin scaffold has been used in stem cell engineering and has been found to be effective for repairing bone, cartilage, ligament, and skin [169, 170]. In addition, it has been especially helpful in achieving cell attachment and growth, but it performs better when conjugated with other materials. Chen et al. reported that the surface modification of silk fibroin with arginyl-glycyl-aspartic acid (RGD) peptide resulted in higher BM stromal cell attachment, cell density, and collagen matrix formation [171]. Likewise, there are other biomaterials, such as hyaluronic acid for alleviating osteoarthritis symptoms, fibrin as a sealant, and alginate for ESC-derived beta cell encapsulation [169].

In general, natural biomaterials contain the RGD binding moieties that aid in the attachment of cells to the culture plate. The initial attachment of cells to a substrate is an essential step in the differentiation of PSC into MSC. Stronger attachment would generally ensures better cellular responses such as proliferation and differentiation.

In the case of PSC to MSC differentiation, it has been shown that PSC differentiating to MSC express high levels of integrins $\alpha 5 \beta 1$, a known protein that binds to integrin. Thus, the use of fibronectin coated dishes were found to enhance the isolation of PSC-derived MSC in-vitro [172].

Although natural biomaterials are effective and efficient in inducing cellular responses, their use in cell and tissue engineering is limited due to some limitations. First, natural materials may not be easily accessible in large quantities and thus are not preferred in upscaled or routinary experiments [173, 174]. Another reason is that they are normally extracted from different living organisms which is a labor-intensive process. Upon extraction, these materials need to be further purified and devoid of contamination. Because of these, natural biomaterials are expensive compared with synthetic materials. Finally, due to the complexity of these full-sized ECM proteins, tuning its mechanical strength for specific purposes is rather inefficient. Modifications to the base material is also often limited [175]. As a result, some scientist shifted towards the development and use of synthetic materials.

Synthetic biomaterials

Apart from natural polymers, synthetic polymers are promising as biomaterials. Synthetic biomaterials are artificially derived polymeric materials that are synthesized by combining small monomeric units, for example, polyethylene glycol (PEG), poly(glycolic acid) [PGA], poly(D,L-lactic acid) [PLA], poly(D,L-lactic acid-co-glycolic acid) (PLGA), poly(3-hydroxybutyrate), and poly(ϵ -caprolactone) (PCL). These monomers are polymerized to form stable structures that can alter cellular activity. Unlike natural biomaterials, synthetic biomaterials are devoid of batch-to-batch variation, mechanically stronger, more durable, and their structures can be modified easily to achieve various porosities and permeabilities [173]. Generally, these materials are unreactive to cells owing to the presence of hydrophilic groups on their surfaces. However, their interaction can be modified by conjugating them with growth factors, proteins, and small molecules to facilitate cell-material interactions. One of the most commonly used conjugates is the RGD peptide, which induces the attachment of cells onto materials owing to the inert binding capacity of integrin on cell surfaces and conjugation the RGD peptide conjugated to the material [176]. However, when selecting a biomaterial to obtain a particular cellular activity, its biocompatibility, biodegradability, and toxicity must be considered carefully.

Polyethylene Glycol PEG is a polyether compound derived from petroleum and used for medical purposes. PEG is a non-ionic, biocompatible, hydrophilic compound that can

be crosslinked using several methods to modify its physicochemical and biological properties. When iMSCs were cultured in PEG diacrylate conjugated with RGD peptide, they efficiently differentiated into valve interstitial cells [177]. Other modifications such as N-cadherin-loaded PEG dimethacrylate hydrogels and poly(*N-isopropyl acrylamide-co*-)PEG supports expansion and differentiation of mESC and hPSCs respectively [178, 179].

Polyvinyl alcohol (PVA) Polyvinyl alcohol (PVA) is another hydrophilic polymer that is prepared through the hydrolysis of polyvinyl acetate or vinyl ester. It is a biocompatible and non-toxic material that requires surface modification for cell adhesion. In a study, PVA-co-itaconic acid hydrogel grafted with fibronectin and oligopeptide derived from the cell ECM led to stable expansion of hPSCs for several passages without any signs of differentiation [180, 181]. The stiffness of the hydrogel was maintained at 25.3 kPa throughout the PSC proliferation test. The stiffness of this range is found to be favorable for stem cell proliferation, but differentiation was observed in hydrogel with higher stiffness. Thus, to increase stiffness of the material, PVA is combined with PEG which helped in the differentiation of hESC toward mesoderm [182].

Poly(lactic acid (PLA)/ Poly(D,L-lactic acid-co-glycolic acid) (PLGA) Poly(lactic acid (PLA) is a hydrophobic, biocompatible, tunable, and non-immunogenic polymer that is commonly used as a scaffold in cell culture [183]. It has been used for MSC cell culture and differentiation. This polymer, however, is normally found blended with other polymers to obtain materials of various strengths, degradation rates, and thermal stabilities.

For example, co-polymerization of PLA with glycolic acid leads to the formation of PLGA copolymer. This copolymer has enhanced material properties that are important for cell and tissue culture. The PGA component imparts degradable properties, whereas the PLA component provides improved cell adhesion, which makes PLGA extremely useful for regulating various cellular activities [184]. In one study, MSC cultured over a PLGA scaffold containing simvastatin led to bone growth when transplanted at a bone defect site [185]. Having the capability for growth factor attachment and to conjugate proteins and small molecules, PLGA has been utilized in differentiating PSCs into MSCs via the controlled release of growth factors specific for MSC differentiation. For instance, the encapsulation of miRNA extracted from active protein kinase A (PKA) expressing ESC in PLGA nanoparticles induces mesoderm differentiation in ESC that further differentiate into MSC [186]. Apart from the type of biomaterials, their design and interaction with cells regulate changes in cellular properties.

Various biomaterial designs and platforms affect cell behavior

Apart from the nature of the starting materials, the spatio-physical presentation of these materials to cells is equally important for designing a biomaterial-based platform to achieve the differentiation of iPSCs to MSCs. The design of a biomaterial imparts different cell behaviors and, in turn, affects cellular processes such as proliferation, differentiation, and survival. Such biomaterial platforms include scaffolds, hydrogels, micro/nanoparticles, and fibers. Depending on the goal of the research, the biomaterial design should be selected carefully to obtain optimum results. For instance, hydrogels provide cells with a 3D microenvironment for survival and contain pores that affect the proliferation and differentiation abilities of cells [187]. Similarly, microparticles are helpful for forming spheroids and avoiding necrotic zone formation within these spheroids [188]. Other important stem cell regulations are discussed in the subsequent paragraphs.

Hydrogels

Hydrogels are 3D polymers composed of hydrophilic compounds, and for this reason, they have the capacity to retain water molecules and swell in response to such retention. These structures are not impermeable solids; instead, they are porous materials that allow water or any solvent to enter within them and expand [189, 190]. Hydrogels are generally composed of polymers with hydroxyl, carboxyl, and amide groups for interaction, hydrophilicity, and gelation. Different hydrogel formation methods have been developed, and each method imparts different physical and chemical properties for various applications. In tissue engineering, hydrogels are primarily prepared from various types of natural (chitosan, collagen, and gelatin) and synthetic (methacrylate, polyvinyl, and polyethylene glycol) materials [191]. By altering these materials and varying their concentrations, researchers can produce different hydrogels with wide ranges of compressibility and elasticity. Hydrogels are known for their biocompatibility and biodegradability, and they demonstrably support cell attachment, drug and growth factor delivery, and cell encapsulation. For these reasons, they have been used to culture various cells, including MSCs and PSCs [192].

One advancement in hydrogel research is the development of composite hydrogels that allow for the formation of different microenvironments through “switching” of the active component. For instance, a hydrogel composed of alginate and collagen can be switched from a composite (alginate–collagen) to a pure collagen hydrogel through subsequent chelation and alginate washing. In this setup, the “switch” from composite to pure alginate induces the differentiation of PSCs into the meso- and ectodermal lineages [193]. Moreover, hydrogels can

be incorporated with microparticles to create composite hydrogels. Dong et al. incorporated stromal-derived factor-1 (SDF-1) and kartogenin into modified chitosan-silk fibroin hydrogel and PLGA particles, respectively, to increase BMMSC proliferation and migration [194].

Porous and solid scaffolds

Scaffolds are 3D constructs that can support cellular processes such as attachment, proliferation, and growth. Although hydrogels can be classified as scaffolds, not all scaffolds are composed of hydrogels, and they can be prepared from various materials, including a wide range of metals, non-metals, fibers, composites, and even decellularized tissues. Similar to hydrogels, scaffolds provide a base structure for cellular attachment and growth. They are more solid and less porous compared to hydrogels [189].

Depending on the purpose, several scaffold preparation methods have been developed to obtain scaffolds of various shapes, compositions, and porosities. The most widely used method is particulate leaching, which involves the dissolution of particles pre-incorporated within the base biomaterial. Upon dissolution, the material retains its shape while leaving permeable “pores.” This method is widely used to obtain porous scaffolds, in addition to other methods such as solvent casting, gas foaming, emulsion templating, fiber formation, electrospraying, and 3D printing [189].

Various scaffolds have been used to regulate the fate of MSCs. For instance, BMMSC migration, adhesion, and osteogenic differentiation were enhanced by using a mesenchymal- and preosteoclast-protein-enriched dental bone matrix scaffold [195]. This scaffold contained proteins that facilitated the migration of cells toward the scaffold. In addition, the natural stiffness of the DBM matrix enhanced osteogenic differentiation. In another study, it was found that a patterned porous honey/silk fibroin scaffold prepared by means of soft lithography enhanced the proliferation of seeded MSCs [196]. A similar result was observed when MSCs were cultured in chitosan calcium polyphosphate and pigeonite scaffold [197].

A few of these scaffolds play an essential role in PSC proliferation and differentiation. A peptide polyvinyl butyral-based polymer scaffold synthesized by means of acrylic polymerization led to effective proliferation and differentiation of PSCs into cells of the mesodermal lineage, and therefore, it can potentially be useful for generating MSCs [198].

Microparticles/nanoparticles

Micro/nanoparticles are small spherical particles that can deliver growth factors or small molecules for cell growth or differentiation. One of the interesting roles of these

particles is stabilizing the growth factors, which lose their efficiency in a free soluble state [199]. In cell and tissue culture, microparticles can be conjugated with the growth factors necessary for various cell functions such as proliferation and differentiation. Moreover, microparticles with varying properties such as stiffness, size, and sensitivity to certain stimuli, such as temperature, can be synthesized to control the release of conjugated growth factors. For instance, a thermo-responsive poly-N-isopropylacrylamide (PNIPAM) loaded with retinoic acid (RA) nanoparticles was used to induce the differentiation of iPSCs into neural cells [200]. The release of RA in these nanoparticles was achieved by changing the incubation temperature of the cells. Similarly, by considering the degradation properties of microparticles, the release of matrilin3 and TGF- β 3 was controlled to achieve effective differentiation of MSCs into chondrocytes [201].

Moreover, microparticles can be used to stabilize certain biomolecules that are rather sensitive such as nucleic acids. In one study, the siRNA of chondrin was stabilized when loaded in crosslinked gelatin microparticles. The stabilized siRNA, in turn, facilitated efficient osteogenic differentiation of MSCs [202, 203]. Microparticles can also be used to provide greater surface area for cell attachment. In one study, functionalization of PLA microparticles with aminopropylmethacrylamide led to increased MSC attachment on its surface and produced larger cell aggregates compared to those produced on non-functionalized surfaces [204]. In another study, an approach to use RGD peptide on microparticles to achieve selective isolation of MSCs from spontaneously differentiated ESCs by using fibronectin was described [172].

Micro/Nanofibers

Another type of biomaterial designs are the micro/nanofibers. These are elongated and extended materials with varying lengths and diameters, and they are useful for creating fibrous scaffolds. Unlike porous scaffolds, fibrous scaffolds are primarily produced by spinning various materials by using methods such as electrospinning, wet spinning, biospinning, interfacial complexation, microfluidic spinning, and melt spinning (extrusion). These different spinning techniques allow for the fabrication of fibers having varying diameters, lengths, elasticities, and overall strengths. In this manner, different types of fibers can be generated for various tissue engineering applications [205].

Apart from the fabrication technique, the mechanical and biochemical properties of fibers are highly dependent on the type of polymer/material used. A few studies have suggested that the combination of two or more polymers can increase the mechanical stability or enhanced the biocompatibility of fibers [206]. In one study, a hybrid

scaffold generated by coating knitted scaffolds with a thin film of poly (ϵ -caprolactone) (group I), poly (D, L-lactide-co-glycolide) nanofibers (group II), and type 1 collagen (group III) enhanced BM stromal cell attachment owing to the optimal mechanical strength of the hybrid scaffold [207].

The surface topography of the spun fibers can contribute to the overall cellular response and development. For instance, MSCs cultured on aligned electrospun poly-L-lactide-co- ϵ -caprolactone fibers exhibited cell proliferation, enhanced spindle morphology, and increased chondrogenic potential as those cultured on randomly oriented fibers [208]. In another study, the alignment of PCL nanofibers affects the differentiation potential of ESCs. Cells outgrow from the differentiated EBs developed along the aligned grooves of nanofibers formed neurites [209]. Indeed, fiber orientations allow cells to change their morphology. This phenomenon can be attributed to changes in the expression of the FAK molecule, actin, and microtubules, which are major regulators of cell attachment and morphology [210].

Existing biomaterial-based systems for differentiation of PSCs into MSCs

The differentiation of PSCs into different cells mostly involves the use of a 2D culture supplied with culture medium and growth factors, which limits the effects of other variables that influence their proliferation and differentiation. The in-vivo cell microenvironment plays an important role in cell functioning. Cells possess different receptors that are activated after their interaction with the components present in these microenvironments. Nowadays, biomaterials are used to mimic the role of the ECMs present in the human body. This allows cells to express genes at higher levels than in cultures without biomaterials [211]. These materials can influence cellular behavior through their controlled degradation rates, cell surface-environment interactions, biomolecule release, and varying stiffness [211]. In particular, PSCs express ECM-associated genes, which affects their differentiation and proliferation [212]. To improve the efficiency of PSCs, processed biomaterials derived from natural sources, such as human and animal proteins, and biocompatible synthetic materials can be used. In addition, various biomaterial-based culture techniques, such as monolayer or 3D culture, have been proven to affect cellular functions.

Natural biomaterials for differentiation of PSCs into MSCs

As described in the previous section, natural biomaterials affect MSC fate and are, therefore, great candidates for differentiating PSCs into MSCs. This is primarily attributed to the presence of cell-binding sites that support MSC induction and generation. For instance, gelatin

and collagen are effective for deriving MSCs from murine iPSCs. Obara et al. cultured iPSCs on collagen- and gelatin-coated plates; after 8 days, fibroblast-like cells were generated, which were sub-cultured again on collagen- and gelatin-coated plates for further maturation, as depicted in Fig. 4a [213]. In another study, Liu et al. cultured PSCs, and their differentiation in collagen type I-coated culture plates increased the EMT of PSCs, which induced their differentiation into MSCs [214]. These researchers cultured dissociated single cells for MSC production because PSCs cultured in colonies did not exhibit the fibroblast-like morphology. In another study, iPSCs cultured on fibrin-coated plates differentiated into MSCs that exhibited enhanced fibronectin secretion and increased migratory activity. This result suggested that the attachment of cells in fibrin induced cytoskeletal changes in iPSCs, directing them toward MSC differentiation [215]. As for 3D systems, the conventional method involves formation of iPSC cell-spheroids called EBs and the attachment of these spheroids onto a surface coated with biomaterials such as gelatin, as described by Frobel, et al. Differentiated iPS-MSCs form cell outgrowths from these spheroids, and these outgrowths are collected for further characterization, as depicted in Fig. 4c [216]. Representative studies on a few of these biomaterial-based 2D and 3D systems are summarized and depicted in Fig. 5, and Fig. 4, respectively.

Synthetic biomaterials for differentiation of PSC into MSCs

The properties of synthetic biomaterials have been shown to be effective for MSC derivation, provided that the material is modified to improve cell attachment for iPSC-derived MSC generation. Diaz et al. established a differentiation protocol for iPSC-derived MSCs by culturing iPSCs on poly(2-[methacryloyloxy] ethyl dimethyl-[3-sulfo-propyl] ammonium hydroxide) (PMEDSAH)-coated culture plates; EBs were formed, which were then transferred onto gelatin-coated plates and differentiated into MSCs in an MSC medium [217]. These cells exhibited enhanced osteogenic properties, indicating changes in the metabolism of the iPSCs owing to the culturing material [218]. Although these materials were used for differentiation, their interactions with cells were not adequately illustrated.

Integrins are among the most studied molecules in terms of cell–ECM interactions. Integrin-associated signaling is a major mediator of biomaterial–cell interactions and is considered an important factor in the differentiation of PSCs [219]. For instance, ESCs cultured on polystyrene well plates engineered with conjugation of peptide ligands of integrin $\alpha 5 \beta 1$ and integrin $\alpha 6 \beta 1$ in mesodermal differentiation medium containing BMP4 expressed early mesodermal markers [220]. These cells exhibited remarkable differentiation potential toward the mesodermal lineage, indicating the possibility of producing MSC-like cells by using this method [221]. Some of these synthetic materials are mentioned in Table 4.

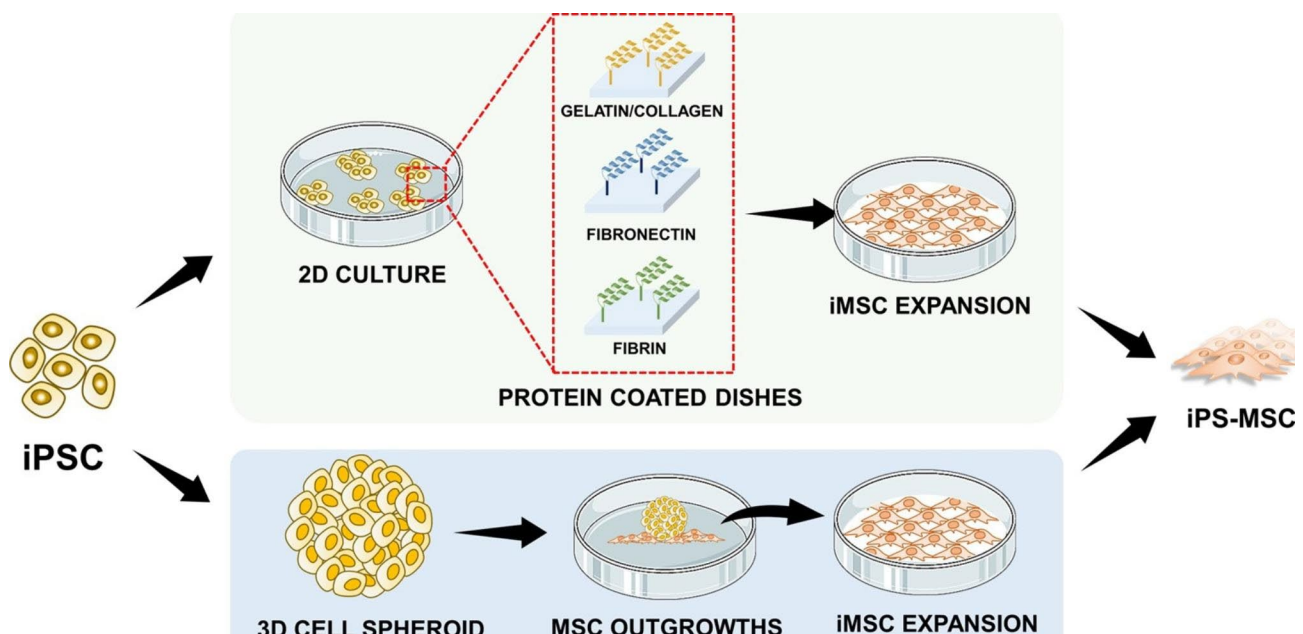
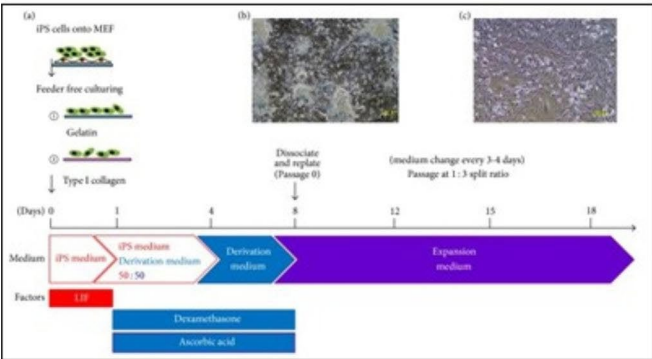
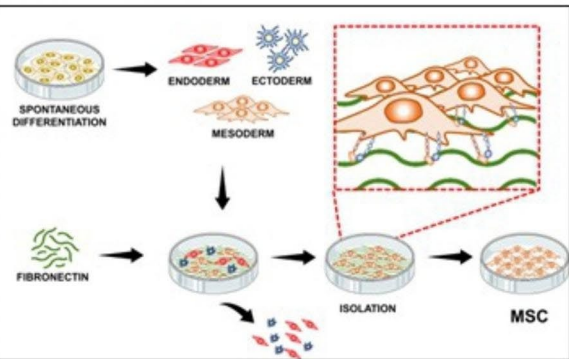


Fig. 4 Representative studies: Existing biomaterial-based technologies for the differentiation of PSC to MSC. (a) Gelatin- and collagen-coated culture dishes as 2D systems for iPS-MSC generation by Obara, et al. [213]. (b) Fibronectin-coated dishes for efficient 2D isolation of iPSC-MSCs from spontaneously differentiated iPS cells by Cha et al. [172] (c) Embryonic bodies cultured in a gelatin-coated dish for generation of iPS-MSC outgrowths by Frobel et al. [216]. Images have been reused with permission

a. Obara et. al.



b. Cha et. al.



c. Frobel et. al.

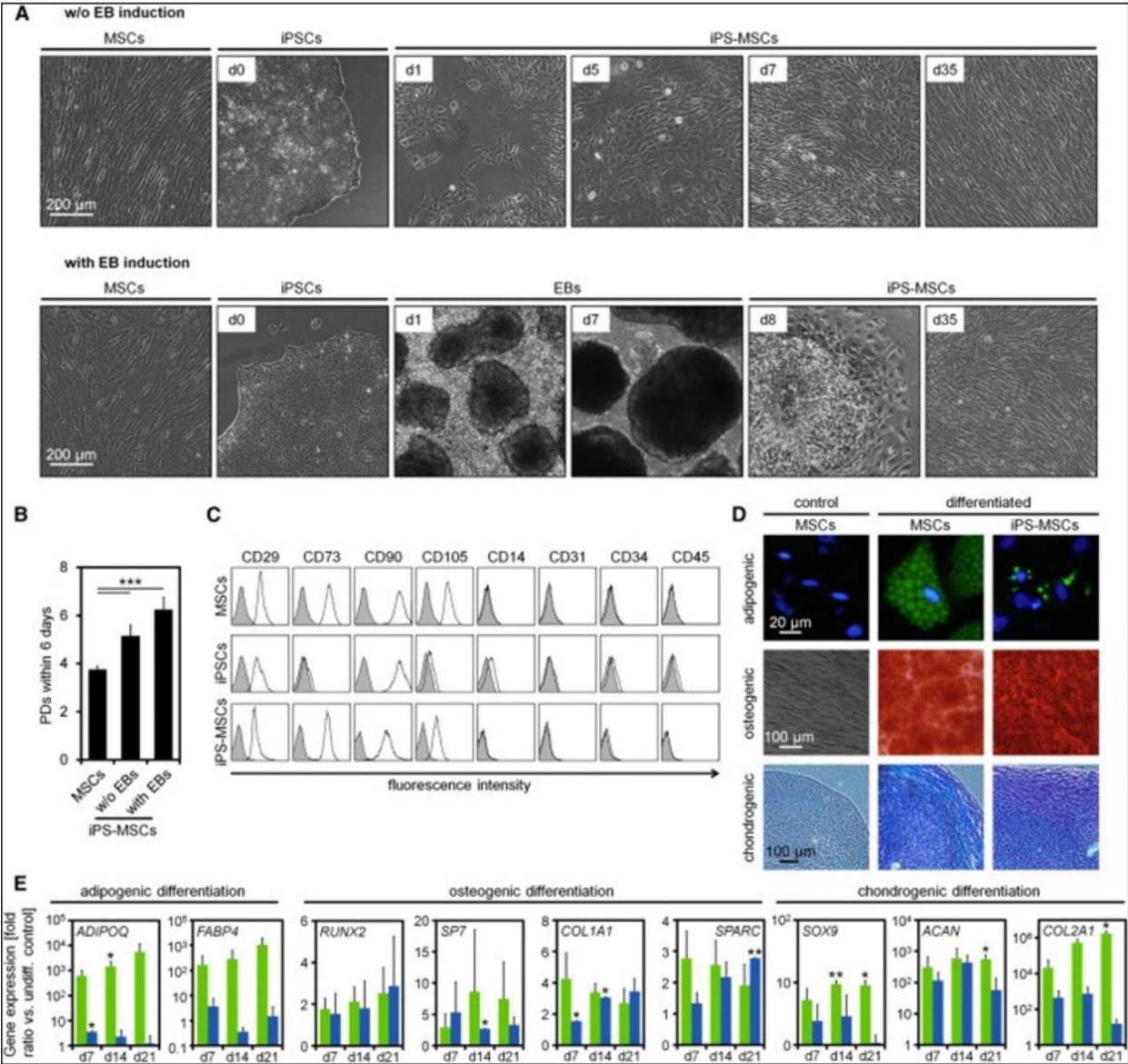


Fig. 5 Conventional 2D and 3D biomaterial-based differentiation of PSC to MSCs. 2D systems generally involve repeated culturing of PSCs in dishes coated with ECM proteins such as gelatin, collagen, fibronectin, and fibrin. 3D systems, by contrast, involve the formation of embryonic bodies (cell spheroids), attachment on coated dishes, and collection of cell outgrowths (MSCs).

Table 4 Various natural and synthetic materials for generation of MSCs from PSCs. Several natural and synthetic materials have been utilized for efficient and effective differentiation of PSCs into MSCs. These materials strongly recapitulate the microenvironment in which MSCs reside in vivo, and therefore, provide a superior platform for MSC generation in in-vitro.

	Biomaterial	Method	Cells used	MSC properties	References
Natural biomaterials	Gelatin and collagen	One-step MSCs derivation method; gelatin and collagen are coated repeatedly for two passages over culture plates	miPSCs	CD73 is expressed; CD90 expression increases with passage, whereas CD105 expression decreases	(213)
	Gelatin	EB are formed for 7 days, and then they are cultured on 0.1% gelatin-coated plates for 2 weeks to attain confluency	hESCs	CD73 and Stro1 markers are expressed; MSCs are successfully differentiated into osteoblasts and adipocytes	(229, 230)
		iPSCs are cultured over 0.1% gelatin for 14 days in MSC medium	iPSCs	All major MSCs markers are expressed with stable passage capacity until 17 passages	(231)
		EBs formed are cultured in 1% gelatin-coated plates; some EBs attach and exhibit outgrowth; the unattached EBs are transferred to another 1% gelatin-coated plate in which MSC-like cells are formed in this culture.	hiPSCs	Both attached and transferred EBs form MSCs, which are termed as aiMSCs and tiMSCs, respectively. aiMSCs exhibit greater CD90 expression than tiMSCs. MSCs markers are expressed in the second passage	(232)
	Collagen type I	Collagen I is used to coat plates, PSCs are cultured over collagen, and repeated passaging is performed	iPSCs and hESCs	After the fourth passage, adult MSCs characteristics are gained; CD90, CD73, and CD105 are strongly expressed. Trilineage potential is observed, but with less adipogenic characteristics	(214)
	Collagen type-IV	EBs are cultured on collagen type IV-coated plate with growth factors to induce expressions of mesodermal and neuroepithelial cells, which are then converted into MSC-like cells in MSC medium for 1 month	hiPSCs	High expression of MSCs markers is observed; differences in paracrine factors can be seen	(233)
	Fibrin	Fibrin is used for differentiation, and both 2D and 3D environment are formed	hiPSCs	All MSCs markers are found; only adipogenic differentiation potential is found; 3D culture yields poorer results	(215)
Synthetic biomaterials	Fibronectin	ESC differentiates spontaneously and are then cultured in fibronectin-coated plates	hESCs	After 7 days, MSC markers are observed, isolation of MSCs from heterogeneous population is achieved; further passages generate mature MSCs with all surface markers and trilineage differentiation potential	(172)
	PMEDSAH	EBs formed from cells are cultured over PMEDSAH-coated plates and subsequently transferred to gelatin-coated plates for MSC generation	hiPSCs	CD90 markers are not observed; high adipogenic, chondrogenic, and osteogenic potentials are noted	(217)
	Polypeptide conjugated gold-coated plates	Integrin ligand-engineered plates are prepared; mesodermal differentiation medium supplemented with BMP4 is used	hESCs	After 48 h, mesodermal markers are observed; further downstream differentiation occurs	(220)

The use of different types of biomaterials is not the only factor that affects the differentiation of iPSCs. Another factor is the system in which the cells are cultured. 2D or 3D culture systems and how they are formed affect the cell proliferation and differentiation efficiencies of iPSCs. These factors regulate cell-cell and cell-matrix interactions, which further affect the cellular activity results.

Two-dimensional (2D) systems for differentiation of PSCs into MSCs

2D cell culture is the conventional method, and it involves the growth and maintenance of cells on flat surfaces. This system provides cells with a substrate for attachment, which is necessary for growth and proliferation. The main drawback of using a 2D culture is that it does not fully recapitulate the physiological conditions of tissues in-vivo, wherein cells are arranged such that they

can interact with the environment in 3D. Although 2D cell cultures may not fully represent tissues *in vivo*, much of the existing molecular biology and medical knowledge has been obtained using 2D cultures.

2D methods play a major role in the proliferation and differentiation of iPSCs into different cells. The use of biomaterials has improved cell maturity and efficacy. Both natural and synthetic materials, as well as hybrid forms, have been used to grow cells. In the 2D culture technique, biomaterials are generally used as a scaffold to provide iPSCs with the traction force needed to differentiate them into different lineages. iPSCs can differentiate into different lineages based on the stiffness, composition, and patterning of the biomaterial scaffold. Soft and stiff substrates support ectodermal and mesodermal lineages, respectively. This defines the effect of biomaterial stiffness on iPSC differentiation in 2D environments [222].

Similarly, a poly(dimethylsiloxane) (PDMS) scaffold with a stiffness of 100 Pa can be used to differentiate iPSCs into neural cells more efficiently than a substrate with a higher stiffness [223]. In another study, cells were induced to express the cardiac gene marker through electrical and mechanical signaling when a PLGA fiber scaffold coated with the conductive polymer polypyrrole was used as the substrate [224]. Similar culturing techniques have led to the efficient generation of MSCs from PSCs. Collagen-, gelatin-, fibrin-, and PMEDSAH-coated plates have been helpful for the conversion of PSCs to MSCs [213–215, 217]. In a study by Cha et al., ESCs cultured on fibronectin-coated plates in a spontaneous differentiation medium efficiently isolated and enhanced MSC-like cells among mixed populations of differentiated cells [172], as depicted in Fig. 4b. The MSCs expressed high levels of $\alpha 5 \beta 1$ integrin, a fibronectin receptor, which indicated that this method efficiently generated MSCs from PSCs. Similarly, it is important to identify the factors that affect cell physiology to produce MSC-like cells. These examples illustrate how 2D culturing methods can affect and even regulate the differentiation processes of cells.

Three-dimensional (3D) systems for the differentiation of PSCs to MSCs

Culturing cells in a 3D environment is an efficient method for replicating the human physiological condition. Cell-cell and cell-ECM interactions are maintained and regulated from every plane in this culture system. There are two methods to create a 3D environment for iPSCs *in vitro*—one involves forming 3D cell aggregates called EBs and the other involves using biomaterials to encapsulate cells. For instance, neuron growth factor-conjugated alginate–chitosan–gelatin hydrogel allowed iPSCs to grow in a 3D environment, thereby enabling them to differentiate into neuron-like cells [225]. In

a study by Zoldan et al., a 3D construct composed of a PLGA hydrogel with varying stiffness enhanced the differentiation efficiency by 50–80-fold [226]. Constructs with higher stiffness promoted the production of mesodermal lineage cells, whereas softer constructs promoted the production of endodermal lineage cells similar to the 2D methods but with higher expression [222]. Although this is an efficient method for iPSC differentiation, several results indicate that 3D methods generally produce smaller cell populations than 2D methods, which can be attributed to the formation of a necrotic zone at the center of the spheroid. This phenomenon has been observed in the 3D culture of fibrin-embedded iPSCs as well. The culture exhibited a heterogeneous population of MSCs with apoptotic clump formation and some fibroblastic colonies among the iPSC population, indicating the influence of limited nutrient supply at different locations in this 3D system [215]. However, this drawback can be resolved by incorporating gelatin microparticles inside the EBs to prevent the formation of necrotic cores [227]. Nevertheless, the biomaterial-based 3D culture of ESCs is a promising approach for inducing their differentiation into the mesodermal lineage [226].

A proper environment that supports efficient differentiation of iPSCs into MSCs must be considered. Little research has been conducted on the biomaterial-assisted differentiation of iPSCs into MSCs. However, the use of 0.1% gelatin in the differentiation of iPSCs into MSCs indicates that the cell surface-expressed $\alpha \nu \beta 3$ and $\alpha 5 \beta 1$ integrins may be the primary factors driving this differentiation [166, 217]. Similarly, 3D environments composed of biomaterials can be developed. A 3D environment created from a PCL nanofiber scaffold with varying stiffness was used by Nam et al. to differentiate embryonic MPCs into different unipotent cells. This technique can be used to differentiate PSCs into MSCs because the stiffness of the scaffold supports mesodermal generation [219, 228].

Future perspective on the biomaterial-based differentiation of iPSCs to MSCs

Recent advances in the differentiation of iPSCs to MSCs mainly include the regulation of molecular signaling pathways through the addition of growth factors or small molecules, whereas few studies have focused on the use of mechanical stimuli. Mechanical signals are provided by the cell's surrounding ECM, and these signals, in turn, affect cell-cell and cell-ECM interactions to induce several molecular processes [234]. Although the exact mechanism by which the ECM regulates cell function has not been fully explored yet, it is hypothesized that mechanical signals affect integrin and laminin proteins expressed on the cell surface, which transfer the signal into the cell's cytoskeleton. This activates the interaction between the Yes-associated protein (YAP) and the transcriptional

coactivator with the PDZ-binding motif (TAZ), which eventually alters the cell's behavior [235, 236].

Depending on the provided mechanical cue, several changes can be observed in cells subjected to mechanical stimuli. In the case of hPSCs, cells transitioned from the pluripotent state to the differentiated state when cultured over a switching hydrogel composed of alginate and collagen, with alginate degradation occurring before collagen degradation [193]. This change in the cell's microenvironment initiated efficient differentiation of hPSCs into ectoderms within 3 days and into mesoderm and endoderm within 5 days. Similarly, modulation of the ECM stiffness can affect the differentiation of hPSCs. In one study, soft ECM (100 and 700 Pa) supported the neuronal differentiation of hPSCs, as indicated by increases in neurogenic and dopaminergic markers within 9 days [223]. Apart from stiffness, neuronal cells from iPSCs were obtained by varying the topographies of PCL through electrospinning [237]. Recent advances in biomaterials and methodologies include the introduction of various mechanical signals to aid the production of different cells from iPSCs [238]. Various methods that use these mechanical cues to generate cardiomyocytes from PSCs have been developed. Puig-Sanvicens et al. reported a biologically inspired self-assembling peptide hydrogel (RAD16-I) that could replace the supplement required for cardiac gene expression, which indicates that the mechanical cues derived from biomaterials can differentiate cells [239]. Similarly, the use of relatively stiffer substrates supports biomaterial in the differentiation process in forming mesoderm lineage cells [240, 241]. In a study by Quinton et al., a PDMS stiffness of 1.7 MPa led to greater mesodermal gene expression than a PDMS stiffness of 3 kPa,

indicating the upregulation of mesodermal markers on relatively stiffer substrates [240]. These studies support the assertion that varying the stiffness and topology of biomaterials induces mechanical cues for the differentiation of hPSCs into mesodermal lineage cells [162]. This procedure can be further extended to produce MSCs from iPSCs because MSCs belong to the mesodermal lineage [221].

Another potential method for MSC production is immobilization of protein on a biomaterial scaffold to initiate PSC differentiation. Immobilizing factors, such as BMP4 and SB431542 and inhibitors of nuclear factor-kappa B (I κ B) kinase (IKK), on PDMS can induce the differentiation of iPSCs into MSCs [144, 159]. Although only a few studies have investigated the role of biomaterials and their impact on iPSC-derived MSC generation, similar studies have indicated the potential of using biomaterials to efficiently produce MSCs from iPSCs. However, proper selection of the base material, biomaterial system (2D versus 3D), correct stiffness, topology, and other MSC-inducing factors should be considered carefully to develop an ideal biomaterial-based system for producing MSCs from iPSCs [242]. The future scope for biomaterial-based MSC production is schematically depicted in Fig. 6.

Conclusion

The use of MSCs for cell therapy and regenerative medicine has increased in recent years owing to the various properties of MSCs that are deemed important in cell and tissue regeneration. MSCs can differentiate into several cell types, such as osteocytes, chondrocytes, and adipocytes. Moreover, MSCs secrete various cytokines

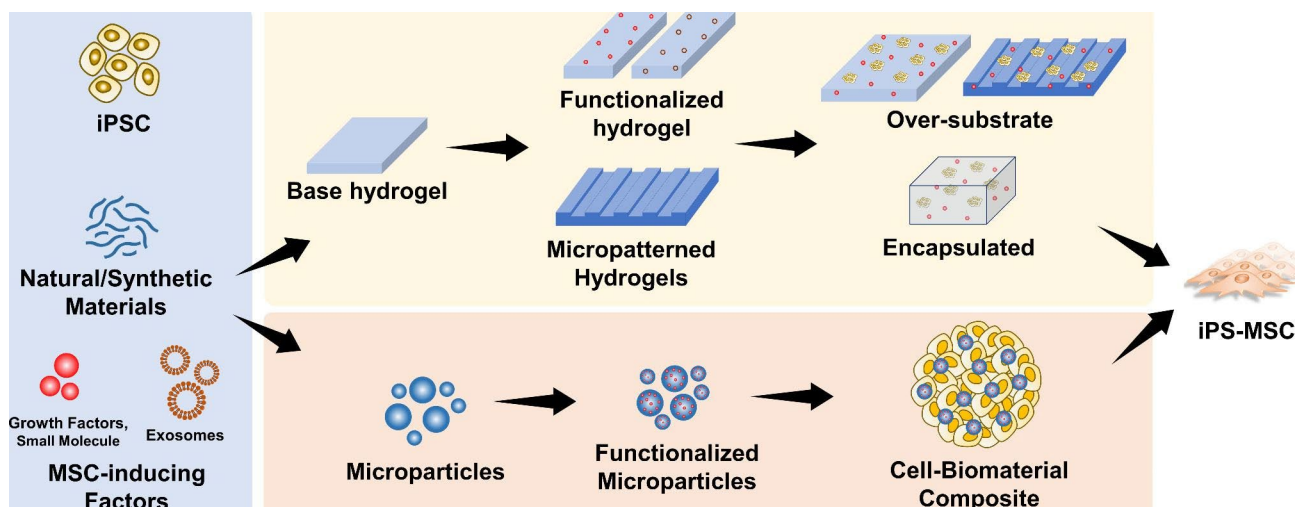


Fig. 6 Future prospects of biomaterial-based MSC generation from PSCs. MSCs can be generated from iPSCs with the help of various biomimicking materials. Several natural and synthetic materials can induce the efficient growth and differentiation of MSCs. Functionalization of these materials through the conjugation of select MSC-inducing growth factors, exosomes, and modifications by varying stiffness and topology represent promising improvements to effectively and efficiently differentiate PSCs into MSCs.

that promote the growth of neighboring cells or regulate immune responses. Owing to these properties, MSCs have been widely used in several clinical trials to treat tissue injuries, such as brain, heart, bone, and cartilage injuries. Their role in treating GVHDs has eased transplantation procedures. These results indicate the promising potential of the use of MSCs to achieve successful transplantations.

MSCs are adult stem cells that are present in almost all tissues of the body. However, variations in their proliferative capacity or differentiation potentials have been observed, and these characteristics are strongly dependent on the tissue and donor from which the cells are isolated. This heterogeneity is the major reason limiting the use of adult MSCs in cell therapy. These limitations can be resolved by deriving MSCs from PSCs. When PSCs are differentiated into MSCs through more defined and regulated procedures, an unlimited supply of clinically relevant MSCs can be obtained. Several strategies, such as growth factor induction, 3D culture, biomaterials, and epigenetics, have been studied. Each technique has unique advantages and disadvantages. However, given that the differentiation of iPSCs into MSCs can be induced via several techniques, it is suggested that a standardized protocol should be developed to realize better and more efficient production of clinically relevant MSCs for use in cell therapy and tissue regeneration.

Abbreviations

AD	Adipose-derived
αMEM	Alpha-minimum essential medium
ADMSC	Adipose-derived mesenchymal stem cell
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BM	Bone marrow
BMMSC	Bone marrow-derived mesenchymal stem cell
BMP	Bone morphogenic protein
CD	Cluster of differentiation
DC	Dendritic cell
DPMSC	Dental pulp-derived mesenchymal stem cell
EB	Embryoid body
ECM	Extracellular matrix
EMT	Epithelial–mesenchymal transition
ESC	Embryonic stem cell
EZH2	Enhancer of zeste homolog 2
FGF2	Fibroblast growth factor 2
GF	Growth factor
GVHD	Graft-versus-host disease
hESC	Human embryonic stem cell
HGF	Hepatocyte growth factor
hPSC	Human pluripotent stem cell
hiPSC	Human induced pluripotent stem cell
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
iMSC	Induced mesenchymal stem cell
iPSC	Induced pluripotent stem cell
MHC	Major histocompatibility complex
MIP-1	Macrophage inflammatory protein-1
miPSC	Mouse induced pluripotent stem cell
MP	Mesodermal progenitor
MPC	Mesenchymal progenitor cell
MSC	Mesenchymal stem cell
MSX2	Muscle segment homeobox 2

NCC	Neural crest cell
NF-κB	Nuclear factor kappa B
PDMS	Poly(dimethylsiloxane)
PCL	Poly(ε-caprolactone)
PEG	Polyethylene glycol
PLA	Poly(lactic acid)
PLGA	Poly(d,l-lactic acid-co-glycolic acid)
PMEDSAH	Poly(2-[methacryloyloxy] ethyl dimethyl-[3-sulfopropyl] ammonium hydroxide)
PPARγ	Peroxisome proliferator-activated receptor gamma
PSC	Pluripotent stem cell
PVA	Polyvinyl alcohol
RA	Retinoic acid
RGD	Arginyl-glycyl-aspartic acid
RUNX-2	Runt-related transcription factor-2
SF	Synovial fluid
SFMSC	Synovial fluid-derived mesenchymal stem cell
SMMSC	Synovial membrane-derived mesenchymal stem cell
Stro-1	Stromal precursor antigen-1
TAZ	Transcriptional coactivator with PDZ-binding motif
TF	Transcription factor
TGF	Transforming growth factor
3D	Three-dimensional
TNFα	Tumor necrosis factor alpha
2D	Two-dimensional
UC	Umbilical cord
UCA	Umbilical cord artery
UCB	Umbilical cord blood
UCMSC	Umbilical cord-derived mesenchymal stem cell
UCV	Umbilical cord vein
VEGF	Vascular endothelial growth factor
VWMSC	Vascular wall mesenchymal stem cell
WJ	Wharton's jelly
WJMSCs	Wharton's jelly-derived mesenchymal stem cells
YAP	Yes-associated protein

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40824-023-00371-0>.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

Not applicable.

Authors' contributions

The review topic was conceived and designed by N.P, H.P, A.B., and S.L. The manuscript was drafted by N.P., J.K., J.J., and Y.A. and it was revised and critically edited by H.P., A.B., and S.L. All authors have read and approved the manuscript.

Funding

This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2022R1A2C3004850, NRF-2019M3A9H1032376).

Data Availability

Not applicable.

Declarations

Competing interests

There are no conflicts to declare.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Received: 1 February 2023 / Accepted: 26 March 2023

Published online: 18 April 2023

References

1. Bianco P, Robey PG, Simmons PJ. Mesenchymal Stem Cells: Revisiting History, Concepts, and Assays. *Cell Stem Cell*. 2008;2(4):313–9.
2. Andrzejewska A, Lukomska B, Janowski M. Concise Review: Mesenchymal Stem Cells: From Roots to Boost. *Stem Cells*. 2019;37(7):855–64.
3. Andrukhov O, Behm C, Blufstein A, Rausch-Fan X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: Implication in disease and tissue regeneration. *World J Stem Cells*. 2019;11(9):604–14.
4. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Exp Mol Med*. 2013;45(11):e54.
5. Berebichez-Fridman R, Montero-Olvera PR. Sources and clinical applications of mesenchymal stem cells: state-of-the-art review. *Sultan Qaboos Univ Med J*. 2018;18:264–77.
6. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8:315–7.
7. Lee WS, Kim HJ, Kim K, Il, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: a phase IIb, Randomized, Placebo-Controlled Clinical Trial. *Stem Cells Transl Med*. 2019;8:504–11.
8. Zhou Y, Yamamoto Y, Xiao Z, Ochiya T. The immunomodulatory functions of mesenchymal Stromal/Stem cells mediated via paracrine activity. *J Clin Med*. 2019;8:1025–42.
9. Pankajakshan D, Agrawal DK. Mesenchymal Stem Cell Paracrine Factors in Vascular Repair and Regeneration. *J Biomed Technol Res*. 2014;1(1).
10. Linero I, Chaparro O. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. *PLoS ONE*. 2014 Sep;8(9):e107001.
11. Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS ONE*. 2014 Dec;26(12):e115963.
12. Wang X, Wang Y, Gou W, Lu Q, Peng J, Lu S. Role of mesenchymal stem cells in bone regeneration and fracture repair: a review. *Int Orthop*. 2013;37:2491–8.
13. Yamanaka S. Pluripotent stem cell-based cell Therapy—Promise and Challenges. *Cell Stem Cell*. 2020;27:523–31.
14. Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. *J Hematol Oncol*. 2021;14:24.
15. 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;4:22–36.
16. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet*. 1970;3:393–403.
17. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al. Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement. *Cytotherapy*. 2005;7:393–5.
18. Lin CS, Xin ZC, Dai J, Lue TF. Commonly used mesenchymal stem cell markers and tracking labels: Limitations and challenges. *Histol Histopathol*. 2013 Sep;28:1109–16.
19. Duff SE, Li C, Garland JM, Kumar S. CD105 is important for angiogenesis: evidence and potential applications. *FASEB J*. 2003;17:984–92.
20. Sauzay C, Voutetakis K, Chatziioannou A, Chevet E, Avril T. CD90/Thy-1, a Cancer-Associated Cell Surface Signaling Molecule. *Front Cell Dev Biol*. 2019;7:66.
21. Rege TA, Hagood JS. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. *FASEB J*. 2006;20:1045–54.
22. Maleki M, Ghanbarvand F, Behvarz MR, Ejtemaei M, Ghadirkhomi E. Comparison of mesenchymal stem cell markers in multiple human adult stem cells. *Int J Stem Cells*. 2014;7:118–26.
23. Li M, Soder R, Abhyankar S, Abdelhakim H, Braun MW, Trinidad CV, Pathak HB, Pessetto Z, Deighan C, Ganguly S, Dawn B, McGuirk J, Dunavin N, Godwin AK. WJMSC-derived small extracellular vesicle enhance T cell suppression through PD-L1. *J Extracell Vesicles*. 2021;10(4):e12067.
24. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. 2006;24:1294–301.
25. Salehinejad P, Moshrefi M, Eslaminejad T. An overview on mesenchymal stem cells derived from extraembryonic tissues: supplement sources and isolation methods. *Stem Cells Cloning*. 2020;13:57–65.
26. Hu L, Yin C, Zhao F, Ali A, Ma J, Qian A. Mesenchymal stem cells: cell fate decision to osteoblast or adipocyte and application in osteoporosis treatment. *Int J Mol Sci*. 2018;19:360–79.
27. Dieudonne FX, Sévère N, Biosse-Duplan M, Weng JJ, Su Y, Marie PJ. Promotion of osteoblast differentiation in mesenchymal cells through cbl-mediated control of STAT5 activity. *Stem Cells*. 2013;31:1340–9.
28. Chen Q, Shou P, Zheng C, Jiang M, Cao G, Yang Q, et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? *Cell Death Differ*. 2016;23:1128–39.
29. Boeuf S, Richter W. Chondrogenesis of mesenchymal stem cells: role of tissue source and inducing factors. *Stem Cell Res Ther*. 2010;1:1–9.
30. Nanda NK, Birch L, Greenberg NM, Prins GS. MHC class I and Class II Molecules are expressed in both human and mouse prostate Tumor Microenvironment. *Prostate*. 2006;66:1275–84.
31. Mattar P, Bieback K. Comparing the Immunomodulatory Properties of Bone Marrow, adipose tissue, and Birth-Associated tissue mesenchymal stromal cells. *Front Immunol*. 2015;6:560–8.
32. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. *Cell Prolif*. 2020;53:e12712.
33. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol*. 2008;8:726–36.
34. Mukai T, Tojo A, Nagamura-Inoue T. Umbilical cord-derived mesenchymal stromal cells contribute to Neuroprotection in neonatal cortical neurons damaged by oxygen-glucose deprivation. *Front Neurol*. 2018;9:466.
35. Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood*. 2002;99:3838–43.
36. Fischbach MA, Bluestone JA, Lim WA. Cell-based therapeutics: the Next Pillar of Medicine. *Sci Transl Med*. 2013;5:179p57.
37. Hmadcha A, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V. Therapeutic potential of mesenchymal stem cells for Cancer Therapy. *Front Bioeng Biotechnol*. 2020;8:43.
38. Wright A, Arthaud-Day ML, Weiss ML. Therapeutic use of mesenchymal stromal cells: the need for inclusive characterization guidelines to accommodate all tissue sources and species. *Front Cell Dev Biol*. 2021;9:66.
39. Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *Int J Rheum Dis*. 2011;14:211–5.
40. Davatchi F, Sadeghi Abdollahi B, Mohyeddin M, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients. *Int J Rheum Dis*. 2016;19:219–25.
41. Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. *Cell Transpl*. 2004;13:595–600.
42. Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, Sentís J, Sánchez A, García-Sancho J. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: two-year follow-up results. *Transplantation*. 2014;97:e66–8.
43. Chahal J, Gómez-Aristizábal A, Shestopaloff K, Bhatt S, Chaboureaud A, Fazio A, et al. Bone marrow mesenchymal stromal cell treatment in patients with Osteoarthritis results in overall improvement in Pain and symptoms and reduces synovial inflammation. *Stem Cells Transl Med*. 2019;8:746–57.
44. Fodor PB, Paulseth SG. Adipose derived stromal cell (ADSC) injections for Pain Management of Osteoarthritis in the human knee Joint. *Aesthet Surg J*. 2016;36:229–36.
45. Pers YM, Rackwitz L, Ferreira R, Pullig O, Delfour C, Barry F, et al. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation Trial. *Stem Cell Translational Medicine*. 2016;5:847–56.

46. Lu L, Dai C, Zhang Z, Du H, Li S, Ye P, et al. Treatment of knee osteoarthritis with intra-articular injection of autologous adipose-derived mesenchymal progenitor cells: a prospective, randomized, double-blind, active-controlled, phase IIb clinical trial. *Stem Cell Res Ther.* 2019;10:143–53.
47. Chen YC, Chang CH. Clinical application of mesenchymal stem cells for cartilage regeneration. *Plast Aesthet Res.* 2020;7:49.
48. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoobar GM. Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. *Clin Oral Implants Res.* 2011;22:251–8.
49. Gómez-Barrena E, Padilla-Eguiluz N, Rosset P, Gebhard F, Hernigou P, Baldini N, Rouard H, Sensebé L, Gonzalo-Daganzo RM, Giordano R, García-Rey E, Cordero-Ampuero J, Rubio-Suárez JC, García-Simón MD, Stanovici J, Ehrnthalter C, Huber-Lang M, Flouzat-Lachaniette CH, Chevallier N, Donati DM, Spazzoli B, Ciapetti G, Fleury S, Fernandez MN, Cabrera JR, Avendaño-Solá C, Montemurro T, Panaitescu C, Veronesi E, Rojewski MT, Lotfi R, Dominici M, Schrezenmeier H, Layrolle P. Early efficacy evaluation of mesenchymal stromal cells (MSC) combined to biomaterials to treat long bone non-unions. *Injury.* 2020;51(Suppl 1):63–S73.
50. Liao LL, Looi QH, Chia WC, Subramaniam T, Ng MH, Law JX. Treatment of spinal cord injury with mesenchymal stem cells. *Cell Biosci.* 2020;10:112.
51. Oh SK, Choi KH, Yoo JY, Kim DY, Kim SJ, Jeon SR. A phase III clinical trial showing limited efficacy of autologous mesenchymal stem cell therapy for spinal cord injury. *Neurosurgery.* 2016;78:436–47.
52. Le Blanc K, Frasson F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *The Lancet.* 2008;371:1579–86.
53. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol.* 2004;94:92–5.
54. Guo J, Lin GS, Bao CY, Hu ZM, Hu MY. Anti-inflammation role for mesenchymal stem cells transplantation in myocardial infarction. *Inflammation.* 2007;30:97–104.
55. Wang T, Zhang J, Liao J, Zhang F, Zhou G. Donor genetic backgrounds contribute to the functional heterogeneity of stem cells and clinical outcomes. *Stem Cells Transl Med.* 2020;9:1495.
56. Sammour I, Somashekar S, Huang J, Batlahally S, Breton M, Valasaki K. The effect of gender on mesenchymal stem cell (MSC) efficacy in neonatal Hyperoxia-Induced Lung Injury. *PLoS ONE.* 2016;11:e0164269.
57. Mushahary D, Spittler A, Kasper C, Weber V, Charwat V. Isolation, cultivation, and characterization of human mesenchymal stem cells. *Cytometry Part A.* 2018;93:19–31.
58. Friedenstien AJ, Chailakhyan RK, Gerasimov U. v. bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. *Cell Prolif.* 1987;20:263–72.
59. Drela K, Stanaszek L, Snioch K, Kuczynska Z, Wrobel M, Sarzynska S et al. Bone marrow-derived from the human femoral shaft as a new source of mesenchymal stem/stromal cells: an alternative cell material for banking and clinical transplantation. *Stem Cells Res Ther.* 2020;11.
60. Maacha S, Sidahmed H, Jacob S, Gentilcore G, Calzone R, Grivel JC, et al. Paracrine mechanisms of mesenchymal stromal cells in Angiogenesis. *Stem Cells Int.* 2020;2020:4356359.
61. Cai M, Shen R, Song L, Lu M, Wang J, Zhao S, et al. Bone marrow mesenchymal stem cells (BM-MSCs) improve heart function in Swine Myocardial Infarction Model through Paracrine Effects. *Sci Rep.* 2016;6:28250.
62. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* 2001;7:211–28.
63. Huang SJ, Fu RH, Shyu WC, Liu SP, Jong GP, Chiu YW, et al. Adipose-derived stem cells: isolation, characterization, and differentiation potential. *Cell Transpl.* 2013;22:701–9.
64. Ghatreh K, Eliyasi M, Alaei S, Saki G. Differentiation potential of adipose tissue-derived mesenchymal stem cells into germ cells with and without growth factors. *Andrologia.* 2021;53:e13892.
65. Markov A, Thangavelu L, Aravindhan S, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. *Stem Cells Res Ther.* 2021;12:192.
66. Tsai AI, Hong HH, Lin WR, Fu JF, Chang CC, Wang IK, Huang WH, Weng CH, Hsu CW, Yen TH. Isolation of mesenchymal stem cells from human deciduous Teeth Pulp. *Biomed Res Int.* 2017;2017:2851906.
67. Ledesma-Martínez E, Mendoza-Núñez VM, Santiago-Osorio E. Mesenchymal stem cells derived from Dental Pulp: a review. *Stem Cells Int.* 2016;2016:4709572.
68. Hagmann S, Rimmele C, Bucur F, Dreher T, Zeifang F, Moradi B, et al. Mesenchymal stromal cells from osteoarthritic synovium are a distinct Population compared to their bone-marrow counterparts regarding surface marker distribution and immunomodulation of allogeneic CD4 + T-Cell cultures. *Stem Cells Int.* 2016;2016:6579463.
69. Zhang Z, Ding Y, Li W, Song B, Yang R. Interleukin-17A- or tumor necrosis factor α -mediated increase in proliferation of T cells cocultured with synovium-derived mesenchymal stem cells in rheumatoid arthritis. *Arthritis Res Ther.* 2013;15:R169.
70. Hatakeyama A, Uchida S, Utsunomiya H, Tsukamoto M, Nakashima H, Nakamura E, et al. Isolation and characterization of synovial mesenchymal stem cell derived from hip joints: a comparative analysis with a matched control knee Group. *Stem Cells Int.* 2017;2017:9312329.
71. Heil JR, Bordon B, Embryology. Umbilical Cord. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557490/>
72. Fong CY, Subramanian A, Gauthaman K, Venugopal J, Biswas A, Ramakrishna S, et al. Human umbilical cord Wharton's Jelly stem cells undergo enhanced chondrogenic differentiation when grown on Nanofibrous Scaffolds and in a sequential two-stage culture medium environment. *Stem Cell Rev Rep.* 2011;8:195–209.
73. Semenova E, Grudniak MP, Machaj EK, Bocian K, Chroscinska-Krawczyk M, Trochonowicz M, et al. Mesenchymal stromal cells from different parts of umbilical cord: Approach to Comparison & characteristics. *Stem Cell Rev Rep.* 2021;17:1780–95.
74. Marino L, Castaldi MA, Rosamilio R, Ragni E, Vitolo R, Fulgione C, et al. Mesenchymal stem cells from the Wharton's Jelly of the human umbilical cord: Biological Properties and therapeutic potential. *Int J Stem Cells.* 2019;12:218–26.
75. Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: their advantages and potential clinical utility. *World J Stem Cells.* 2014;6:195–202.
76. Mebarki M, Abadie C, Larghero J, Cras A. Human umbilical cord-derived mesenchymal stem/stromal cells: a promising candidate for the development of advanced therapy medicinal products. *Stem Cell Res Ther.* 2021;12:152–61.
77. Bharti D, Shivakumar SB, Park JK, Ullah I, Subbarao RB, Park JS, et al. Comparative analysis of human Wharton's jelly mesenchymal stem cells derived from different parts of the same umbilical cord. *Cell Tissue Res.* 2018;372:51–65.
78. Chen JY, Mou XZ, Du XC, Xiang C. Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins. *Asian Pac J Trop Med.* 2015;8:739–46.
79. Doi H, Kitajima Y, Luo L, Yan C, Tateishi S, Ono Y, et al. Potency of umbilical cord blood- and Wharton's jelly-derived mesenchymal stem cells for scarless wound healing. *Sci Rep.* 2016;6:18844.
80. Shauki S, Gaafar T, Erfan H, Khateeb E, El SA, El, Hawary R, El. Immunomodulatory effects of umbilical cord-derived mesenchymal stem cells. *Microbiol Immunol.* 2015;59:348–56.
81. Luo G, Cheng W, He W, Wang X, Tan J, Fitzgerald M, et al. Promotion of cutaneous wound healing by local application of mesenchymal stem cells derived from human umbilical cord blood. *Wound Repair and Regeneration.* 2010;18:506–13.
82. Talwadekar MD, Kale VP, Limaye LS. Placenta-derived mesenchymal stem cells possess better immunoregulatory properties compared to their cord-derived counterparts—a paired sample study. *Sci Rep.* 2015;5:15784.
83. Zhou J, Wang D, Liang T, Guo Q, Zhang G. Amniotic fluid-derived mesenchymal stem cells: characteristics and therapeutic applications. *Arch Gynecol Obstet.* 2014;290:223–31.
84. Siddesh SE, Gowda DM, Jain R, Gulati A, Patil GS, Anudeep TC et al. Placenta-derived mesenchymal stem cells (P-MSCs) for COVID-19 pneumonia—a regenerative dogma. *Stem Cell Investig [J].* 2021;8.
85. Yamaguchi S, Horie N, Satoh K, Ishikawa T, Mori T, Maeda H, et al. Age of donor of human mesenchymal stem cells affects structural and functional recovery after cell therapy following ischaemic stroke. *J Cereb Blood Flow Metabolism [Internet].* 2018;38:1199–212.
86. Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B. Aging of mesenchymal stem cell in vitro. *BMC Cell Biol.* 2006;7:14.
87. Yosupov N, Haimov H, Juodzbalys G. Mobilization. Isolation and Characterization of Stem Cells from Peripheral Blood: a Systematic Review. *J Oral Maxillofac Res.* 2017;8(1).

88. McDaniel JS, Antebi B, Pilia M, Hurtgen BJ, Belenkiy S, Necsoiu C et al. Quantitative Assessment of Optimal Bone Marrow Site for the Isolation of Porcine Mesenchymal Stem Cells. *Stem Cells Int*. 2017; 2017:1836960.
89. Yang YHK, Ogando CR, Wang See C, Chang TY, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. *Stem Cell Res Ther*. 2018;9:131–44.
90. Aslan H, Zilberman Y, Kandel L, Liebergall M, Oskouian RJ, Gazit D, et al. Osteogenic differentiation of Noncultured Immunoisolated Bone Marrow-Derived CD105 + cells. *Stem Cells*. 2006;24:1728–37.
91. Levi B, Wan DC, Goltzsch JP, Hyun J, Januszyk M, Montoro D, et al. CD105 protein depletion enhances human adipose-derived stromal cell osteogenesis through reduction of transforming growth factor β 1 (TGF- β 1) signaling. *J Biol Chem*. 2011;286:39497–509.
92. Lv FJ, Tuan RS, Cheung KMC, Leung VYL. Concise Review: the surface markers and identity of human mesenchymal stem cells. *Stem Cells*. 2014;32:1408–19.
93. Pérez-Silos V, Camacho-Morales A, Fuentes-Mera L. Mesenchymal stem cells subpopulations: application for Orthopedic Regenerative Medicine. *Stem Cells Int*. 2016;2016:3187491.
94. Calabrese G, Giuffrida R, Lo Furno D, Parrinello NL, Forte S, Gulino R, et al. Potential effect of CD271 on human mesenchymal stromal cell proliferation and differentiation. *Int J Mol Sci*. 2015;16:15609–24.
95. Hermida-Gómez T, Fuentes-Boquete I, Gimeno-Longas MJ, Muiños-López E, Díaz-Prado S, De Toro FJ, et al. Bone marrow cells immunomagnetically selected for CD271 + antigen promote in vitro the repair of articular cartilage defects. *Tissue Eng Part A*. 2011;17:1169–79.
96. Pontikoglou C, Langonné A, Ba MA, Varin A, Rosset P, Charbord P, et al. CD200 expression in human cultured bone marrow mesenchymal stem cells is induced by pro-osteogenic and pro - inflammatory cues. *J Cell Mol Med*. 2016;20:655–65.
97. Gaebel R, Furlani D, Sorg H, Polchow B, Frank J, Bieback K, et al. Cell origin of human mesenchymal stem cells determines a different Healing performance in Cardiac Regeneration. *PLoS ONE*. 2011;6:e15652.
98. Pham LH, Pham LH, Vu NB, Pham P, Van. The subpopulation of CD105 negative mesenchymal stem cells show strong immunomodulation capacity compared to CD105 positive mesenchymal stem cells. *Biomedical Res Therapy*. 2019;6:3131–40.
99. Sobacchi C, Palagano E, Villa A, Menale C. Soluble factors on stage to direct mesenchymal stem cells fate. *Front Bioeng Biotechnol*. 2017;5:532.
100. Kan C, Chen L, Hu Y, Lu H, Li Y, Kessler JA, et al. Microenvironmental factors that regulate mesenchymal stem cells: Lessons learned from the study of heterotopic ossification. *Histol Histopathol*. 2017;32:977–85.
101. Liu J, Gao J, Liang Z, Gao C, Niu Q, Wu F, et al. Mesenchymal stem cells and their microenvironment. *Stem Cell Research and Therapy*. 2022;13:429.
102. Tan L, Liu X, Dou H, Hou Y. Characteristics and regulation of mesenchymal stem cell plasticity by the microenvironment — specific factors involved in the regulation of MSC plasticity. *Genes and Diseases*. 2022;9:296–309.
103. Glenn JD. Mesenchymal stem cells: emerging mechanisms of immunomodulation and therapy. *World J Stem Cells [Internet]*. 2014;6:526.
104. Song L, Webb NE, Song Y, Tuan RS. Identification and functional analysis of candidate genes regulating Mesenchymal Stem Cell Self-Renewal and Multipotency. *Stem Cells [Internet]*. 2006;24:1707–18.
105. Bensidhoum M, Chapel A, Francois S, Demarquay C, Mazurier C, Fouillard L, et al. Homing of in vitro expanded Stro-1- or Stro-1 + human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. *Blood*. 2004;103:3313–9.
106. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. *Injury*. 2005;36:1392–404.
107. Xian L, Wu X, Pang L, Lou M, Rosen CJ, Qiu T, et al. Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nat Med*. 2012;18:1095–101.
108. Hu C, Wu Y, Wan Y, Wang Q, Song J. Introduction of hIGF-1 gene into bone marrow stromal cells and its effects on the cell's biological behaviors. *Cell Transpl*. 2008;17:1067–81.
109. Youssef A, Aboalola D, Han VKM. The roles of insulin-like growth factors in mesenchymal stem cell niche. *Stem Cells International*. 2017;2017:9453108.
110. Feng X, Huang D, Lu X, Feng G, Xing J, Lu J, et al. Insulin-like growth factor 1 can promote proliferation and osteogenic differentiation of human dental pulp stem cells via mTOR pathway. *Dev Growth Differ*. 2014;56:615–24.
111. Youssef A, Iosef C, Han VKM. Low-oxygen tension and igf-i promote proliferation and multipotency of placental mesenchymal stem cells (PMSCs) from different gestations via distinct signaling pathways. *Endocrinology*. 2014;155:1386–97.
112. Crane JL, Cao X. Bone marrow mesenchymal stem cells and TGF- β signaling in bone remodeling. *J Clin Invest*. 2014;124:466–72.
113. Dubon MJ, Yu J, Choi S, Park KS. Transforming growth factor β induces bone marrow mesenchymal stem cell migration via noncanonical signals and N-cadherin. *J Cell Physiol*. 2018;233:201–13.
114. Li SN, Wu JF. TGF- β /SMAD signaling regulation of mesenchymal stem cells in adipocyte commitment. *Stem Cell Res Ther*. 2020;11:1–10.
115. Takam Kamga P, Bazzoni R, Dal Collo G, Cassaro A, Tanasi I, Russignan A, et al. The role of notch and wnt signaling in MSC Communication in Normal and Leukemic Bone Marrow Niche. *Front Cell Dev Biology*. 2021;8:599276.
116. Jian H, Shen X, Liu I, Semenov M, He X, Wang XF. Smad3-dependent nuclear translocation of β -catenin is required for TGF- β 1- induced proliferation of bone marrow-derived adult human mesenchymal stem cells. *Genes Dev*. 2006;20:666–74.
117. Blahnova VH, Filova E, Dankova J, Rampichova M. Combinations of growth factors for human mesenchymal stem cell proliferation and osteogenic differentiation. *Bone Joint Res*. 2020;9:412–20.
118. Nguyen VT, Canciani B, Cirillo F, Anastasia L, Peretti GM, Mangiavini L. Effect of chemically Induced Hypoxia on osteogenic and angiogenic differentiation of bone marrow mesenchymal stem cells and human umbilical vein endothelial cells in direct coculture. *Cells*. 2020;9:757.
119. Boskey AL. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. *Bonekey Rep*. 2013;2:447.
120. Dзамukova M, Brunner TM, Miotla-Zarebska J, et al. Mechanical forces couple bone matrix mineralization with inhibition of angiogenesis to limit adolescent bone growth. *Nat Commun*. 2022;13:3059.
121. Hankenson KD, Dishowitz M, Gray C, Schenker M. Angiogenesis in bone regeneration. *Injury*. 2011;42:556–61.
122. Bartold M, Gronthos S, Haynes D, Ivanovski S. Mesenchymal stem cells and biologic factors leading to bone formation. *J Clin Periodontol*. 2019;46:12–32.
123. Somaiah C, Kumar A, Mawrie D, Sharma A, Patil SD, Bhattacharyya J et al. Collagen Promotes Higher Adhesion, Survival and Proliferation of Mesenchymal Stem Cells. *PLoS One*. 2015 Dec 1;10:e0145068.
124. Lin HY, Tsai CC, Chen LL, Chiou SH, Wang YJ, Hung SC. Fibronectin and laminin promote differentiation of human mesenchymal stem cells into insulin producing cells through activating akt and ERK. *J Biomed Sci*. 2010;17:56.
125. Peng KY, Liu YH, Li YW, Yen BL, Yen ML. Extracellular matrix protein laminin enhances mesenchymal stem cell (MSC) paracrine function through α v β 3/CD61 integrin to reduce cardiomyocyte apoptosis. *J Cell Mol Med*. 2017;21:1572–83.
126. Lee MN, Hwang HS, Oh SH, Roshanzadeh A, Kim JW, Song JH, et al. Elevated extracellular calcium ions promote proliferation and migration of mesenchymal stem cells via increasing osteopontin expression. *Exp Mol Med*. 2018;50:142.
127. Viti F, Landini M, Mezzelani A, Petecchia L, Milanese L, Scaglione S. Osteogenic differentiation of MSC through calcium signaling activation: Transcriptomics and functional analysis. *PLoS ONE*. 2016;11:e0148173.
128. Vinchi F, Mendelson A, Yazdanbakhsh K, An X. Uncovering the Bone Marrow Microenvironment Cell by CellHemisphere [Internet]. 2019;3.
129. Peci F, Dekker L, Pagliaro A, van Bostel R, Nierkens S, Belderbos M. The cellular composition and function of the bone marrow niche after allogeneic hematopoietic cell transplantation. *Bone Marrow Transplantation* 2022. 2022;57:9. [Internet].
130. Bello AB, Park H, Lee SH. Current approaches in biomaterial-based hematopoietic stem cell niches. *Acta Biomater*. 2018;72:1–15.
131. Sun M, Chi G, Xu J, Tan Y, Xu J, Lv S, et al. Extracellular matrix stiffness controls osteogenic differentiation of mesenchymal stem cells mediated by integrin α 5. *Stem Cell Res Ther*. 2018;9:52.
132. Navarrete RO, Lee EM, Smith K, Hyzy SL, Doroudi M, Williams JK, et al. Substrate stiffness controls osteoblastic and chondrocytic differentiation of mesenchymal stem cells without exogenous stimuli. *PLoS ONE*. 2017;12:e0170312.
133. Ferlin KM, Prendergast ME, Miller ML, Kaplan DS, Fisher JP. Influence of 3D printed porous architecture on mesenchymal stem cell enrichment and differentiation. *Acta Biomater*. 2016;32:161–9.
134. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006;126:677–89.
135. Abagnale G, Steger M, Nguyen VH, Hersch N, Sechi A, Jousen S, et al. Surface topography enhances differentiation of mesenchymal stem cells towards osteogenic and adipogenic lineages. *Biomaterials*. 2015;61:316–26.

136. Chaudhary JK, Rath PC. Microgrooved-surface topography enhances cellular division and proliferation of mouse bone marrow-derived mesenchymal stem cells. *PLoS ONE*. 2017;12:e0182128.
137. Meng X, Su RJ, Baylink DJ, Neises A, Kiroyan JB, Lee WYW, et al. Rapid and efficient reprogramming of human fetal and adult blood CD34+ cells into mesenchymal stem cells with a single factor. *Cell Res*. 2013;23:658–72.
138. Yu P, Pan G, Yu J, Thomson JA. FGF2 sustains NANOG and switches the outcome of BMP4-induced human embryonic stem cell differentiation. *Cell Stem Cell*. 2011;8:326–34.
139. Li E, Zhang Z, Jiang B, Yan L, Park JW, Xu RH. Generation of mesenchymal stem cells from human embryonic stem cells in a complete serum-free condition. *Int J Biol Sci*. 2018;14:1901–9.
140. Wang X, Lazorchak AS, Song L, Li E, Zhang Z, Jiang B, et al. Immune modulatory mesenchymal stem cells derived from human embryonic stem cells through a trophoblast-like stage. *Stem Cells*. 2016;34:380–91.
141. Wu Q, Zhang L, Su P, Lei X, Liu X, Wang H, et al. MSX2 mediates entry of human pluripotent stem cells into mesoderm by simultaneously suppressing SOX2 and activating NODAL signaling. *Cell Res*. 2015;25:1314–32.
142. Zhang L, Wang H, Liu C, Wu Q, Su P, Wu D, et al. MSX2 initiates and accelerates mesenchymal Stem/Stromal cell specification of hPSCs by regulating TWIST1 and PRAME. *Stem Cell Reports*. 2018;11:497–513.
143. Chen YS, Pelekanos RA, Ellis RL, Horne R, Wolvetang EJ, Fisk NM. Small Molecule Mesengenic induction of Human Induced Pluripotent Stem cells to Generate Mesenchymal Stem/Stromal cells. *Stem Cell Translational Medicine*. 2012;1:83–95.
144. Mahmood A, Harkness L, Schröder HD, Abdallah BM, Kassem M. Enhanced differentiation of human embryonic stem cells to mesenchymal progenitors by inhibition of TGF- β /activin/nodal signaling using SB-431542. *J Bone Miner Res*. 2010;25:1216–33.
145. Sánchez L, Gutierrez-Aranda I, Ligerio G, Rubio R, Muñoz-López M, García-Pérez JL, et al. Enrichment of human ESC-derived multipotent mesenchymal stem cells with immunosuppressive and anti-inflammatory properties capable to protect against experimental inflammatory bowel disease. *Stem Cells*. 2011;29:251–62.
146. Fukuta M, Nakai Y, Kirino K, Nakagawa M, Sekiguchi K, Nagata S, et al. Derivation of mesenchymal stromal cells from pluripotent stem cells through a neural crest lineage using small molecule compounds with defined media. *PLoS ONE*. 2014;9:e112291.
147. Lin Y, Chen G. Embryoid body formation from human pluripotent stem cells in chemically defined E8 media. 2014. In: *StemBook*. Cambridge (MA): Harvard Stem Cell Institute; 2008–. PMID: 28211653.
148. Kurosawa H. Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. *J Biosci Bioeng*. 2007;103:389–98.
149. Bratt-Leal AM, Carpenedo RL, McDevitt TC. Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation. *Biotechnol Prog*. 2009;25:43–51.
150. Stover AE, Schwartz PH. The Generation of Embryoid Bodies from Feeder-Based or feeder-free human pluripotent stem cell cultures. *Methods Mol Biol*. 2011;767:391–8.
151. Hwang NS, Varghese S, Lee HJ, Zhang Z, Ye Z, Bae J et al. In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells. *Proceedings of the National Academy of Sciences*. 2008;105:20641–6.
152. Lee EJ, Lee HN, Kang HJ, Kim KH, Hur J, Cho HJ, et al. Novel embryoid body-based method to Derive Mesenchymal Stem cells from human embryonic stem cells. *Tissue Eng Part A*. 2010;16:705–15.
153. Frobel J, Hemeda H, Lenz M, Abagnale G, Jousen S, Denecke B, et al. Epigenetic rejuvenation of mesenchymal stromal cells derived from Induced Pluripotent Stem cells. *Stem Cell Reports*. 2014;3:414–22.
154. Barberi T, Willis LM, Succi ND, Studer L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med*. 2005;2:554–60.
155. Karlsson C, Emanuelsson K, Wessberg F, Kajic K, Axell MZ, Eriksson PS, et al. Human embryonic stem cell-derived mesenchymal progenitors—potential in regenerative medicine. *Stem Cell Res*. 2009;3:39–50.
156. Steens J, Zuk M, Benchellal M, Bornemann L, Teichweyde N, Hess J, et al. In Vitro Generation of Vascular Wall-Resident Multipotent Stem cells of Mesenchymal Nature from Murine Induced Pluripotent Stem cells. *Stem Cell Reports*. 2017;8:919–32.
157. Yu Y, Deng P, Yu B, Szymanski JM, Aghaloo T, Hong C, et al. Inhibition of EZH2 promotes human embryonic stem cell differentiation into mesoderm by reducing H3K27me3. *Stem Cell Reports*. 2017;9:752–61.
158. Richter A, Valdimarsdottir L, Hrafnkelsdottir HE, Runarsson JF, Omarsdottir AR, van Oostwaard DW, et al. BMP4 promotes EMT and mesodermal commitment in human embryonic stem cells via SLUG and MSX2. *Stem Cells*. 2014;32:636–48.
159. Deng P, Zhou C, Alvarez R, Hong C, Wang CY. Inhibition of IKK/NF- κ B signaling enhances differentiation of mesenchymal stromal cells from human embryonic stem cells. *Stem Cell Reports*. 2016;6:456–65.
160. K A, A K JY, ChuJianlin, QinYu ChanAnn, et al. Mesenchymal stem cell Population Derived from Human pluripotent stem cells displays potent Immunomodulatory and Therapeutic Properties. *Stem Cells Dev*. 2014;23:1611–24.
161. Boyd NL, Robbins KR, Dhara SK, West FD, Stice SL. Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells. *Tissue Eng Part A*. 2009;15:1897–907.
162. Bertucci TB, Dai G. Biomaterial Engineering for Controlling Pluripotent Stem Cell Fate. *Stem Cells Int*. 2018;2018:9068203.
163. Brovold M, Almeida JL, Pla-Palacin I, Sainz-Arnal P, Sánchez-Romero N, Rivas JJ, et al. Naturally-derived biomaterials for tissue Engineering Applications. *Adv Exp Med Biol*. 2018;1077:421–49.
164. Park YH, Yun JI, Han NR, Park HJ, Ahn JY, Kim C, et al. Mass production of early-stage bone-marrow-derived mesenchymal stem cells of rat using gelatin-coated matrix. *Biomed Res Int*. 2013;2013:347618.
165. Krishna OD, Jha AK, Jia X, Kiick KL. Integrin-mediated adhesion and proliferation of human MCs elicited by a Hydroxyproline-Lacking, collagen-like peptide. *Biomaterials*. 2011;32:6412–24.
166. Davidenko N, Schuster CF, Bax DV, Farndale RW, Hamaia S, Best SM, et al. Evaluation of cell binding to collagen and gelatin: a study of the effect of 2D and 3D architecture and surface chemistry. *J Mater Sci Mater Med*. 2016;27:148.
167. Xia H, Liang C, Luo P, Huang J, He J, Wang Z, et al. Pericellular collagen i coating for enhanced homing and chondrogenic differentiation of mesenchymal stem cells in direct intra-articular injection. *Stem Cell Res Ther*. 2018;9:1–12.
168. Li GY, Pukunaga S, Takenouchi K, Nakamura F. Comparative study of the physiological properties of collagen, gelatin and collagen hydrolysate as cosmetic materials. *Int J Cosmet Sci*. 2005;27:101–6.
169. Wang Y, Kim HJ, Vunjak-Novakovic G, Kaplan DL. Stem cell-based tissue engineering with silk biomaterials. *Biomaterials*. 2006 Dec;27:6064–82.
170. Zuluaga-Vélez A, Quintero-Martinez A, Orozco LM, Sepúlveda-Arias JC. Silk fibroin nanocomposites as tissue engineering scaffolds – A systematic review. *Biomed Pharmacother*. 2021;141:111924.
171. Chen J, Altman GH, Karageorgiou V, Horan R, Collette A, Volloch V, et al. Human bone marrow stromal cell and ligament fibroblast responses on RGD-modified silk fibers. *J Biomed Mater Res A*. 2003;67:559–70.
172. Cha BH, Kim JS, Bello A, Lee GH, Kim DH, Kim BJ, et al. Efficient isolation and Enrichment of Mesenchymal Stem cells from human embryonic stem cells by utilizing the Interaction between Integrin α 5 β 1 and fibronectin. *Adv Sci*. 2020;7:2001365.
173. Reddy MSB, Ponnammam D, Choudhary R, Sadasivuni KK. A comparative review of natural and synthetic biopolymer composite scaffolds. *Polym (Basel)*. 2021;13:1105.
174. Ige OO, Umoru LE, Aribio S. Natural Products: a minefield of Biomaterials. *ISRN Mater Sci*. 2012;2012:983062.
175. Ricapito NG, Ghobril C, Zhang H, Grinstaff MW, Putnam D. Synthetic biomaterials from metabolically derived synthons. *Chem Rev*. 2016;116:2664–704.
176. Bellis SL. Advantages of RGD peptides for directing cell association with biomaterials. *Biomaterials*. 2011;32:4205–10.
177. Nachlas ALY, Li S, Jha R, Singh M, Xu C, Davis ME. Human iPSC-derived mesenchymal stem cells matured into valve interstitial-like cells using PEGDA hydrogels. *Acta Biomater*. 2018;71:235–46.
178. Lim HJ, Mosley MC, Kurosu Y, Smith Callahan LA. Concentration dependent survival and neural differentiation of murine embryonic stem cells cultured on polyethylene glycol dimethacrylate hydrogels possessing a continuous concentration gradient of n-cadherin derived peptide his-ala-val-asp-ile. *Acta Biomater*. 2017;56:153–60.
179. Adil MM, Rodrigues GMC, Kulkarni RU, Rao AT, Chernavsky NE, Miller EW, et al. Efficient generation of hPSC-derived midbrain dopaminergic neurons in a fully defined, scalable, 3D biomaterial platform. *Sci Rep*. 2017;7:1–11.
180. Sung TC, Li HF, Higuchi A, Ling QD, Yang JS, Tseng YC, Pan CP, Alarfaj AA, Munusamy MA, Kumar S, Hsu ST, Murugan K. Human Pluripotent Stem Cell Culture on Polyvinyl Alcohol-Co-Itaconic Acid Hydrogels with Varying Stiffness Under Xeno-Free Conditions. *J Vis Exp*. 2018;(132):57314.
181. Muduli S, Chen LH, Li MP, Heish ZW, Liu CH, Kumar S, et al. Stem cell culture on polyvinyl alcohol hydrogels having different elasticity and immobilized with ECM-derived oligopeptides. *J Polym Eng*. 2017;37:647–60.

182. Wang Z, Numada A, Wagai F, Oda Y, Eiraku M. Spatial cell fate manipulation of human pluripotent stem cells by controlling the microenvironment using photocurable hydrogel. *bioRxiv*. 2022;<https://doi.org/10.1101/2022.10.02.510557>.
183. Casalini T, Rossi F, Castrovinci A, Perale G. A perspective on polylactic acid-based polymers use for nanoparticles synthesis and applications. *Front Bioeng Biotechnol*. 2019;7:259.
184. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polym (Basel)*. 2011;3:1377–97.
185. Mendes D, Domingues JA, Hausen MA, Cattani SMM, Aragones A, Oliveira ALR, et al. Study of mesenchymal stem cells cultured on a poly(Lactic-co-Glycolic Acid) Scaffold Containing Simvastatin for Bone Healing. *J Appl Biomater Funct Mater*. 2017;15:133–41.
186. Minakawa T, Matoba T, Ishidate F, Fujiwara TK, Takehana S, Tabata Y, et al. Extracellular vesicles synchronize cellular phenotypes of differentiating cells. *J Extracell Vesicles*. 2021;10(11):e12147.
187. Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol Bioeng*. 2009;103:655–63.
188. Pinto B, Henriques AC, Silva PMA, Bousbaa H. Three-dimensional spheroids as in vitro preclinical models for cancer research. *Pharmaceutics*. 2020;12:1186.
189. Park Y, Huh KM, Kang SW. Applications of biomaterials in 3d cell culture and contributions of 3d cell culture to drug development and basic biomedical research. *Int J Mol Sci*. 2021;22:22491.
190. El-Sherbiny IM, Yacoub MH. Hydrogel scaffolds for tissue engineering: Progress and challenges. *Glob Cardiol Sci Pract*. 2013;2013:316–42.
191. Ahmed EM. Hydrogel. Preparation, characterization, and applications: a review. *J Adv Res*. 2015;6:105–21.
192. Chai Q, Jiao Y, Yu X. Hydrogels for biomedical applications: their characteristics and the mechanisms behind them. *Gels*. 2017;3:6.
193. Dixon JE, Shah DA, Rogers C, Hall S, Weston N, Parmenter CDJ, et al. Combined hydrogels that switch human pluripotent stem cells from self-renewal to differentiation. *Proc Natl Acad Sci U S A*. 2014;111:5580–5.
194. Dong Y, Liu Y, Chen Y, Sun X, Zhang L, Zhang Z, et al. Spatiotemporal regulation of endogenous MSCs using a functional injectable hydrogel system for cartilage regeneration. *NPG Asia Mater*. 2021;13:71.
195. Dong R, Bai Y, Dai J, et al. Engineered scaffolds based on mesenchymal stem cells/preosteoclasts extracellular matrix promote bone regeneration. *J Tissue Eng*. 2020;11. <https://doi.org/10.1177/2041731420926918>.
196. Mukhopadhyay A, Das A, Mukherjee S, Rajput M, Gope A, Chaudhary A, et al. Improved mesenchymal stem cell proliferation, differentiation, epithelial transition, and restrained senescence on Hierarchically Patterned Porous Honey Silk Fibroin Scaffolds. *ACS Appl Bio Mater*. 2021;4:4328–44.
197. Dhivya S, Keshav Narayan A, Logith Kumar R, Viji Chandran S, Vairamani M, Selvamurugan N. Proliferation and differentiation of mesenchymal stem cells on scaffolds containing chitosan, calcium polyphosphate and pigeonite for bone tissue engineering. *Cell Prolif*. 2018;51:e12408.
198. Shimizu E, Iguchi H, Le MNT, Nakamura Y, Kobayashi D, Arai Y, et al. A chemically-defined plastic scaffold for the xeno-free production of human pluripotent stem cells. *Sci Rep*. 2022;12:2516.
199. Wang Z, Wang Z, Lu WW, Zhen W, Yang D, Peng S. Novel biomaterial strategies for controlled growth factor delivery for biomedical applications. *NPG Asia Materials*. 2017;9:e435.
200. Seo HI, Cho AN, Jang J, Kim DW, Cho SW, Chung BG. Thermo-responsive polymeric nanoparticles for enhancing neuronal differentiation of human induced pluripotent stem cells. *Nanomedicine*. 2015;11:1861–9.
201. Bello AB, Kim Y, Park S, Muttigi MS, Kim J, Park H, et al. Matrilin3/TGFβ3 gelatin microparticles promote chondrogenesis, prevent hypertrophy, and induce paracrine release in MSC spheroid for disc regeneration. *NPJ Regen Med*. 2021;6:50.
202. Raja MAG, Katas H, Wen TJ. Stability, intracellular delivery, and release of siRNA from chitosan nanoparticles using different cross-linkers. *PLoS ONE*. 2015;10:e0128963.
203. Hinkelmann S, Springwald AH, Starke A, Kalwa H, Wölk C, Hacker MC. Microtissues from mesenchymal stem cells and siRNA-loaded cross-linked gelatin microparticles for bone regeneration. *Mater Today Bio*. 2021;13:10019.
204. Alvarez-Paino M, Amer MH, Nasir A, Cuzzucoli Crucitti V, Thorpe J, Burroughs L. Polymer microparticles with defined Surface Chemistry and Topography mediate the formation of stem cell aggregates and cardiomyocyte function. *ACS Appl Mater Interfaces*. 2019;11:34560–74.
205. Tamayol A, Akbari M, Annabi N, Paul A, Khademhosseini A, Juncker D. Fiber-based tissue engineering: Progress, challenges, and opportunities. *Biotechnol Adv*. 2013;31:669–87.
206. Dai W, Kawazoe N, Lin X, Dong J, Chen G. The influence of structural design of PLGA/collagen hybrid scaffolds in cartilage tissue engineering. *Biomaterials*. 2010;31:2141–52.
207. Sahoo S, Cho-Hong JG, Siew-Lok T. Development of hybrid polymer scaffolds for potential applications in ligament and tendon tissue engineering. *Biomed Mater*. 2007;2:169–73.
208. Kadir ND, Yang Z, Hassan A, Denslin V, Lee EH. Electrospun fibers enhanced the paracrine signaling of mesenchymal stem cells for cartilage regeneration. *Stem Cell Res Ther*. 2021;12:100.
209. Xie J, Willerth SM, Li X, Macewan MR, Rader A, Sakiyama-Elbert SE, et al. The differentiation of embryonic stem cells seeded on electrospun nanofibers into neural lineages. *Biomaterials*. 2009;30:354–62.
210. Jo J, Abdi Nansa S, Kim DH. Molecular regulators of Cellular Mechanoadaptation at cell–material interfaces. *Front Bioeng Biotechnol*. 2020;8:608569.
211. Tang M, Chen W, Liu J, Weir MD, Cheng L, Xu HHK. Human Induced Pluripotent Stem cell-derived mesenchymal stem cell seeding on calcium phosphate Scaffold for Bone Regeneration. *Tissue Eng Part A*. 2014;20:1295–305.
212. Liu H, Lin J, Roy K. Effect of 3D scaffold and dynamic culture condition on the global gene expression profile of mouse embryonic stem cells. *Biomaterials*. 2006;27:5978–89.
213. Obara C, Takizawa K, Tomiyama K, Hazawa M, Sotome-Nakamura A, Gotoh T, et al. Differentiation and Molecular Properties of mesenchymal stem cells derived from Murine Induced Pluripotent stem cells derived on gelatin or collagen. *Stem Cells Int*. 2016;2016:9013089.
214. Liu Y, Goldberg AJ, Dennis JE, Gronowicz GA, Kuhn LT. One-step derivation of mesenchymal stem cell (MSC)-Like cells from human pluripotent stem cells on a Fibrillar Collagen Coating. *PLoS ONE*. 2012;7:e33225.
215. Goetzke R, Keijder H, Franzen J, Ostrowska A, Nüchtern S, Mela P, et al. Differentiation of Induced Pluripotent Stem cells towards mesenchymal stromal cells is hampered by culture in 3D hydrogels. *Sci Rep*. 2019;9:15578–89.
216. Frobel J, Hemeda H, Lenz M, Abagnale G, Jousen S, Denecke B, et al. Epigenetic rejuvenation of mesenchymal stromal cells derived from induced pluripotent stem cells. *Stem Cell Reports*. 2014;3(3):414–22.
217. Villa-Diaz LG, Brown SE, Liu Y, Ross A, Lahann J, Parent JM, et al. Derivation of functional mesenchymal stem cells from human induced pluripotent stem cells cultured on synthetic polymer substrates. *Stem Cells*. 2012;30:1174–81.
218. Zhang N, Kohn DH. Using Polymeric materials to Control Stem Cell Behavior for tissue regeneration. *Birth Defects Research C Embryo Today*. 2012;96:63–81.
219. Perez RA, Choi SJ, Han CM, Kim JJ, Shim H, Leong KW, et al. Biomaterials control of pluripotent stem cell fate for regenerative therapy. *Prog Mater Sci*. 2016;82:234–93.
220. Liu Y, Wang X, Kaufman DS, Shen W. A synthetic substrate to support early mesodermal differentiation of human embryonic stem cells. *Biomaterials*. 2011;32:8058–66.
221. Vodyanik MA, Yu J, Zhang X, Tian S, Stewart R, Thomson JA, et al. A mesoderm-derived precursor for mesenchymal stem and endothelial cells. *Cell Stem Cell*. 2010;7:718–29.
222. Maldonado M, Luu RJ, Ico G, Ospina A, Myung D, Shih HP, et al. Lineage- and developmental stage-specific mechanomodulation of induced pluripotent stem cell differentiation. *Stem Cell Res Ther*. 2017;8:1–7.
223. Keung AJ, Asuri P, Kumar S, Schaffer DV. Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. *Integr Biology*. 2012;4:1049–58.
224. Gelmi A, Cieslar-Pobuda A, De Muinck E, Los M, Rafat M, Jager EWH, et al. Direct mechanical stimulation of stem cells: a beating electromechanically active Scaffold for Cardiac tissue Engineering. *Adv Healthc Mater*. 2016;5:1471–80.
225. Kuo YC, Wang CC. Guided differentiation of induced pluripotent stem cells into neuronal lineage in alginate–chitosan–gelatin hydrogels with surface neuron growth factor. *Colloids Surf B Biointerfaces*. 2013;104:194–9.
226. Zoldan J, Karagiannis ED, Lee CY, Anderson DG, Langer R, Levenberg S. The influence of scaffold elasticity on germ layer specification of human embryonic stem cells. *Biomaterials*. 2011;32:9612.
227. Hayashi K, Tabata Y. Preparation of stem cell aggregates with gelatin microspheres to enhance biological functions. *Acta Biomater*. 2011;7:2797–803.
228. Nam J, Johnson J, John, Lannutti J, Agarwal S. Modulation of embryonic mesenchymal progenitor cell differentiation via control over pure mechanical Modulus in Electrospun Nanofibers. *Acta Biomater*. 2011;7:1516–24.
229. Brown SE, Tong W, Krebsbach PH. The derivation of mesenchymal stem cells from human embryonic stem cells. *Cells Tissues Organs*. 2009;189:256–60.

230. Guzzo RM, Gibson J, Xu RH, Lee FY, Drissi H. Efficient differentiation of human iPSC-derived mesenchymal stem cells to chondroprogenitor cells. *J Cell Biochem*. 2013;114:480–90.
231. Zou L, Luo Y, Chen M, Wang G, Ding M, Petersen CC, et al. A simple method for deriving functional MSCs and applied for osteogenesis in 3D scaffolds. *Sci Rep*. 2013;3:1–10.
232. Sheyn D, Ben-David S, Shapiro G, De Mel S, Bez M, Ornelas L, et al. Human Induced Pluripotent stem cells differentiate into functional mesenchymal stem cells and repair bone defects. *Stem Cells Transl Med*. 2016;5:1447–60.
233. Eto S, Goto M, Soga M, Kaneko Y, Uehara Y, Mizuta H, et al. Mesenchymal stem cells derived from human iPS cells via mesoderm and neuroepithelium have different features and therapeutic potentials. *PLoS ONE*. 2018;13:e0200790.
234. Kumar A, Placone JK, Engler AJ. Understanding the extracellular forces that determine cell fate and maintenance. *Dev (Cambridge)*. 2017;144:4261–70.
235. Goetzke R, Sechi A, De Laporte L, Neuss S, Wagner W. Why the impact of mechanical stimuli on stem cells remains a challenge. *Cell Mol Life Sci*. 2018;75:3297–312.
236. Naqvi SM, McNamara LM. Stem cell mechanobiology and the role of Biomaterials in governing mechanotransduction and Matrix Production for tissue regeneration. *Front Bioeng Biotechnol*. 2020;8:597661.
237. Mohtaram NK, Ko J, King C, Sun L, Muller N, Jun MBG, et al. Electrospun biomaterial scaffolds with varied topographies for neuronal differentiation of human-induced pluripotent stem cells. *J Biomed Mater Res A*. 2015;103:2591–601.
238. Jang HK, Kim BS. Modulation of Stem Cell differentiation with Biomaterials. *Int J Stem Cells*. 2010;3:80–4.
239. Puig-Sanvicens VAC, Semino CE, zur Nieden NI. Cardiac differentiation potential of human induced pluripotent stem cells in a 3D self-assembling peptide scaffold. *Differentiation*. 2015;90:101–10.
240. Smith Q, Chan XY, Carmo AM, Trempel M, Saunders M, Gerecht S. Compliant substratum guides endothelial commitment from human pluripotent stem cells. *Sci Adv*. 2017;3:e1602883.
241. Evans ND, Minelli C, Gentleman E, LaPointe V, Patankar SN, Kallivretaki M, et al. Substrate stiffness affects early differentiation events in embryonic stem cells. *Eur Cell Mater*. 2009;18:1–13.
242. Langhans SA. Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. *Front Pharmacol*. 2018;9:6.

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