The Influence of Intervertebral Disc Microenvironment on the Biological Behavior of Engrafted Mesenchymal Stem Cells

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Intervertebral disc degeneration is the main cause of low back pain. Traditional treatment methods cannot repair degenerated intervertebral disc tissue. The emergence of stem cell therapy makes it possible to regenerate and repair degenerated intervertebral disc tissue. At present, mesenchymal stem cells are the most studied, and different types of mesenchymal stem cells have their own characteristics. However, due to the harsh and complex internal microenvironment of the intervertebral disc, it will affect the biological behaviors of the implanted mesenchymal stem cells, such as viability, proliferation, migration, and chondrogenic differentiation, thereby affecting the therapeutic effect. This review is aimed at summarizing the influence of each intervertebral disc microenvironmental factor on the biological behavior of mesenchymal stem cells, so as to provide new ideas for using tissue engineering technology to assist stem cells to overcome the influence of the microenvironment in the future.

1. Introduction

Low back pain (LBP) is a common health concern worldwide. Studies have shown that the prevalence of LBP ranges between 1.4% and 20%, which is the main reason for years lived with disability counts and puts a heavy economic burden on the patients and society [1–3]. The diseases that present with symptoms of LBP are mainly spinal degenerative diseases, such as discogenic LBP and lumbar disc herniation [4], and their occurrence is closely related to intervertebral disc (IVD) degeneration (IDD) [5, 6].

Due to the varying severity of clinical manifestations in patients, step-by-step therapy is often used in clinical practice. Most patients tend to opt for conservative treatments due to mild symptoms and short course of the disease, including bed rest, oral painkillers, and functional exercises. Interventional treatments, such as epidural injections and percutaneous intradiscal therapies, are generally performed if conservative treatments fail [7, 8]. With severe symptoms or ineffective conservative and interventional treatments, surgery is often recommended. Although surgical treatments can effectively relieve pain, they may cause complications such as infection, nerve damage, large blood vessel damage, and adjacent segment degeneration due to improper operation or care, which further damage the body of the patients [9, 10]. In addition, neither conservative treatments, interventional treatments, nor surgical treatments can repair the degenerated IVD tissue. Traditional treatments are in a dilemma, and a new treatment is urgently needed to induce repair of degenerated disc tissue.

In recent years, with the successful application of stem cell therapy in neurological diseases, cardiovascular diseases, diabetes, and other fields [11–13], researchers have begun to explore the therapeutic effect of stem cells in IDD. Stem cells come from a wide range of sources, among which mesenchymal stem cells (MSCs) are the most studied. There are several studies that have shown disc height restoration, T2-weighted signal intensity on MRI, improved histology, extracellular matrix (ECM) gene expression, and pain relief after transplantation of MSCs in animal models and
preliminary human clinical trials [14–19]. However, the optimal type of MSCs for the treatment of intervertebral disc degeneration has not yet been determined. In addition, IVD is an avascular tissue with a harsh and complex internal microenvironment, which has been shown to have an impact on the biological behavior of the implanted MSCs, which in turn affects the therapeutic effect [20, 21]. Some studies even reported that MSCs did not demonstrate superior benefits for IDD compared with other therapies, and microenvironmental factors were an important reason for the failure of stem cell repair [22, 23]. These contradictory results suggest that the successful translation of stem cell transplantation for IDD into clinical efficacy remains a formidable challenge. Therefore, in order to accurately utilize tissue engineering technology to improve the therapeutic effect of MSCs in the treatment of IDD, we need to better understand the influence of the internal microenvironment of the IVD on the biological behavior of the implanted stem cells. This review is aimed at outlining the advantages and disadvantages of the different types of MSCs for IDD treatment and focuses on the impact of various microenvironmental factors on the biological behavior of MSC viability, proliferation, migration, and chondrogenic differentiation.

2. IDD and MSCs

2.1. The Basic Structure of the IVD. The IVD is composed of an outer annulus fibrosus (AF), a nucleus pulposus (NP) in the middle, and cartilage endplates (CEPs) at the upper and lower ends. The AF is arranged in layers with the NP as the center. The outer layer is composed of collagen-I, and collagen-II increases gradually from outside to inside, and all the collagen-II reaches the NP [24]. NP is the main structure of IVD, which is mainly composed of NP cells and ECM. The main components of ECM are collagen-II, proteoglycans, and other matrix proteins, the first two of which are two markers of chondrogenesis [25]. Proteoglycans are a special class of glycoproteins consisting of a core protein and one or more glycosaminoglycans, with multiple proteoglycans linked to hyaluronic acid (HA) chains to form aggrecans. Aggrecan provides a high-level charge density that maintains water within the NP by creating a high osmotic pressure [26]. Since the NP is highly hydrated, the IVD tissue has a high degree of elasticity and is able to withstand and buffer normal spinal biological stress. In the absence of a normal ECM, disc degeneration will occur.

2.2. Application of Endogenous and Exogenous MSCs in the Treatment of IDD. The most important pathophysiological changes in IDD are reduced NP cells and enhanced ECM catabolism. Therefore, it becomes a reasonable therapeutic strategy to replenish lost NP cells and ECM by implanting MSCs into degenerative IVD tissues. NP cells are chondrocyte-like cells, and there is no specific and generally accepted marker to differentiate them from chondrocytes [21, 27]. MSCs are cells with self-replication, renewal, multidirectional differentiation, and paracrine potential, which mainly achieve the purpose of treating IDD through two major mechanisms. On the one hand, MSCs have the ability to differentiate into chondroblasts, so they can proliferate and differentiate into NP cells and synthesize ECM after implantation [28, 29]. On the other hand, MSCs have paracrine capacity. They can not only secrete a variety of growth factors to increase the activity of resident IVD cells and promote the synthesis of ECM but also secrete certain anti-inflammatory cytokines to regulate the inflammatory response in degenerated IVD and delay the process of IDD [30–32]. Since the process of transplanting MSCs into degenerative IVDs is an invasive procedure, researchers have increasingly focused on the possibility of homing exogenous and endogenous MSCs into IVDs [33]. The homing of MSCs refers to a process in which cells are recruited from their original niche to injured or pathological tissue, which can be induced by a variety of growth factors and chemokines [33, 34]. However, this endogenous repair strategy for IDD is still in the preclinical research stage and requires further research (Figure 1).

There are many types of MSCs that are used for IDD treatment. According to different tissue sources, MSCs can be divided into two categories: endogenous and exogenous MSCs. Endogenous MSCs refer to IVD-derived stem cells isolated and cultured from normal and degenerative IVD tissue. According to the different localization, endogenous MSCs can be divided into three types: NP-derived MSCs (NP-MSCs), AF-derived MSCs (AF-MSCs), and CEP-derived MSCs (CE-MSCs) [35, 36]. Due to the basic characteristics of MSCs, most endogenous MSCs express MSC-like surface markers including CD73, CD90, and CD105 on the cell surface but do not express CD14, CD19, CD34, CD45, and HLA-DR [36–40]. Some researchers believe that stem cells may have their own specific expression of cell surface markers. For example, stromal cell antigen-1 (STRO-1) may be a specific cell surface marker of CE-MSCs [39, 41], and tyrosine kinase receptor-2 (Tie-2) and disialoganglioside-2 (GD-2) can be used as specific cell surface markers of NP-MSCs [42]. However, further research is needed on the cell surface specific markers of endogenous MSCs. In addition, endogenous MSCs have the ability of osteogenic, adipogenic, and chondrogenic differentiation. In terms of chondrogenic differentiation, some studies have shown that CE-MSCs have stronger differentiation ability, followed by AF-MSCs and NP-MSCs that are lower [36, 43]. The most common types of exogenous MSCs are bone marrow-derived MSCs (BM-MSCs) and adipose-derived MSCs (AD-MSCs), a few are umbilical cord-derived MSCs (UC-MSCs), and there are other rare types of MSCs, such as placental-derived MSCs (PMSCs), amniotic fluid-derived MSCs (AF-MSCs), amniotic membrane-derived MSCs (AM-MSCs), synovial-derived MSCs (SM-MSCs), and peripheral blood-derived MSCs (PB-MSCs). These different tissue-derived MSCs have their own advantages and disadvantages when applied to IDD treatment (Table 1) [36, 40, 44–51].

Besides the direct application of MSCs for the treatment of IDD, some researchers have also focused on the indirect use of MSCs, among which extracellular vesicles (EVs) have attracted attention [52]. EVs are lipid bilayer-surrounded vesicles that are incapable of replicating and do not contain a functional nucleus. In terms of their biogenesis, release pathways, size, content, and function, EVs can be divided
into three main subtypes: exosomes, microvesicles (MV), and apoptotic bodies [53]. Exosomes are paracrine cell communication vesicles with a diameter ranging from 30 to 120 nm, which can carry a variety of biologically active molecules such as mRNA, microRNA (miRNA), protein, and lipid, and have great potential in cell-free therapy [54–56]. Compared with other cell types, MSCs can produce more exosomes through the paracrine pathway [57]. The results have shown that MSC-Exos can delay or even reverse the degenerative process of IVD by maintaining ECM homeostasis, inhibiting apoptosis of NP cells, antioxidative, and anti-inflammatory effects, although the exact underlying mechanisms have not been fully elucidated [58–61]. MSC-derived exosomes (MSC-Exos) have unique advantages in the treatment of IDD when compared with MSCs. Firstly, MSC-Exos do not have the associated risks of immunoreactivity and differentiation into unwanted cells, since they are not cells [62]. Secondly, genetic modification of exosomes to express specific ligands allows them to serve as vehicles for targeted drug delivery [63, 64]. Although MSC-Exos are a promising treatment for IDD, many challenges remain before their clinical application. For example, there are currently no standardized protocols for exosomes isolation, and detection methods with high sensitivity and specificity for the therapeutic efficacy of MSC-Exos are lacking [65, 66]. Furthermore, the optimal route of administration of MSC-Exos remains unclear [67, 68]. These challenges remain to be addressed by further research.

### 3. Influence of the IVD Microenvironmental Factors on MSCs

Physiologically, the IVD microenvironment is characterized by hypoxia, nutrient deficiency, acidity, hypertonicity, and
mechanical loading. During the degeneration of the IVD, its internal microenvironment further deteriorates, and mechanical overload, inflammatory cytokines, and protease accumulation also occur [69, 70]. These microenvironmental factors not only affect the resident IVD cells but also have an impact on the biological behavior of the implanted MSCs. The effects of different microenvironmental factors on MSCs are not completely consistent, and the tolerance of the different types of MSCs to the IVD microenvironment is also different. Next, the effects of the following six microenvironmental factors on the biological behavior of engrafted MSCs are described (Figure 2).

### 3.1. Hypoxia

The partial pressure of oxygen in normal adult IVD can be reduced from 10% in the lateral annulus region to 1% in the central region and is always in a hypoxic state [71]. As IVD degenerates, its partial pressure of oxygen may drop even lower. In general, the therapeutic potential of MSCs is investigated under conventional normoxic conditions. Therefore, MSCs transplanted into degenerative discs are in a hypoxic environment. Compared with normoxic conditions, hypoxia can promote the viability, proliferation, and migration of BMSCs [72–75] and can also inhibit senescence and maintain the stemness of BMSCs through down-regulation of E2A-p21 by HIF-1α-Twist pathway [73, 74]. However, other researchers observed that hypoxia inhibited the migration of BMSCs through hypoxia-inducible factor-1α (HIF-1α) and RhoA-mediated pathways [76].

In addition, multiple studies have demonstrated that hypoxia can reduce the inhibitory effect of interleukin-1β (IL-1β) on the chondrogenic differentiation of BMSCs [77], improve the quality of the generated ECM [77, 78], and promote the differentiation of BMSCs to the NP-like phenotype [78–80]. HIF-1α is a key mediator of the beneficial effects of hypoxic environment on chondrogenic differentiation of MSCs [81]. These results support the enhancement of the ability of BMSCs to repair degenerative IVD by hypoxic preconditioning [75, 82].

Although hypoxic environment has also been shown to promote the migration [83] and chondrogenic differentiation [83–85] of AD-MSCs, the results of hypoxia in terms of their viability and proliferation are still controversial. Most studies have shown that hypoxia enhanced the viability and proliferation rate of AD-MSCs [83, 86–88]. Compared with BMSCs, AD-MSCs had a higher tolerance to hypoxia, serum-free and oxidative stress condition [89]. Further research by a team found that HIF-1α promotes the proliferation of AD-MSCs by interacting with basic fibroblast growth factor and vascular endothelial growth factor under hypoxic conditions [90]. However, there are also studies showing conflicting results. Chung et al. [91] found that hypoxia at 1% and 5% oxygen tension negatively affected both the proliferation rate and osteogenic differentiation of AD-MSCs. The results of Li et al. [44] showed that hypoxia with 2% oxygen tension inhibited the viability and proliferation of AD-MSCs and NP-MSCs but promoted the chondrogenic differentiation of cells, and NP-MSCs exhibited more potent biological activity. He et al. [92] found that hypoxia stimulates autophagy via the HIF-1α signaling pathway, thereby increasing NP-MSC resistance to hydrostatic pressure. These findings highlight the beneficial role of hypoxia in stem cell-based IVD regeneration, thereby providing a
Table 2: Effects of hypoxia on the biological behavior of MSCs.

| Cell sources | Year | Team | Journal | Results | Reference |
|--------------|------|------|---------|---------|-----------|
| BMSCs        | 2004 | Risbud et al. | Spine | Hypoxia (2% O<sub>2</sub>) and TGF-β1 induced the differentiation of BMSCs to a NP-like phenotype. | [78] |
|              | 2006 | Grayson et al. | J Cell Physiol | Hypoxia (2% O<sub>2</sub>) enhanced BMSC viability, proliferation, and expression of stemness genes (Oct4 and Rex-1) compared with normoxia. | [72] |
|              | 2007 | Grayson et al. | Biochem Biophys Res Commun | Hypoxia (2% O<sub>2</sub>) promoted the proliferation of BMSCs and maintained their multilineage capabilities compared with normoxia. | [73] |
|              | 2008 | Kanichai et al. | J Cell Physiol | Hypoxia (2% O<sub>2</sub>) promoted the chondrogenic differentiation of BMSCs compared with normoxia. | [81] |
|              | 2009 | Felka et al. | Osteoarthritis Cartilage | Hypoxia (2% O<sub>2</sub>) reduced the negative effect of IL-1β on chondrogenic differentiation of BMSCs. | [77] |
|              | 2011 | Tsai et al. | Blood | Hypoxia (1% O<sub>2</sub>) promoted BMSC proliferation and maintained their chondrogenic differentiation potential compared with normoxia. | [74] |
|              | 2011 | Müller et al. | Cell Transplant | Hypoxia (4% O<sub>2</sub>) promoted chondrogenic differentiation of BMSCs compared with normoxia. | [79] |
|              | 2011 | Stoyanov et al. | Eur Cell Mater | Hypoxia (2% O<sub>2</sub>) and GDF5 (100 ng/mL) promoted the differentiation of BMSCs to a NP-like phenotype. | [80] |
|              | 2011 | Raheja et al. | Cell Biol Int | Hypoxia (1% O<sub>2</sub>) inhibited BMSC migration compared with normoxia. | [76] |
|              | 2018 | Wang et al. | Stem Cells Int | Hypoxic preconditioning (CoCl<sub>2</sub>, 100 μM, 24 h) enhanced BMSC viability, migration, and expression of aggrecan and collagen-II but inhibited their proliferation. | [75] |
|              | 2021 | Peck et al. | Cartilage | Hypoxic preconditioning (2% O<sub>2</sub>) promoted BMSC viability and ECM production. | [82] |
| AD-MSCs      | 2012 | Chung et al. | Res Vet Sci | Hypoxia (1% and 5% O<sub>2</sub>) inhibited the proliferation of AD-MSCs and BMSCs compared with normoxia, and AD-MSCs exhibited higher proliferative potential than BMSCs. | [91] |
|              | 2013 | Portron et al. | PLoS One | Hypoxic preconditioning (5% O<sub>2</sub>) enhanced the chondrogenic differentiation of AD-MSCs in vitro but not in vivo. | [84] |
|              | 2014 | Choi et al. | Journal of Asian Scientific Research | Hypoxia (2% O<sub>2</sub>) promoted AD-MSC viability and proliferation compared with normoxia. | [86] |
|              | 2015 | Fotia et al. | Cytotechnology | Hypoxia (1% O<sub>2</sub>) promotes AD-MSC proliferation and expression of stemness genes (Nanog and Sox-2) compared with normoxia. | [87] |
|              | 2018 | Takahashi et al. | Cell Transplant | Hypoxia (1% O<sub>2</sub>) inhibited AD-MSC viability but promoted their proliferation compared with normoxia. | [89] |
|              | 2019 | Deng et al. | J Cell Physiol | Hypoxia (5% O<sub>2</sub>) promoted AD-MSC proliferation and chondrogenic differentiation potential compared with normoxia. | [90] |
|              | 2020 | Hwang et al. | Tissue Eng Regen Med | Hypoxic preconditioning (1% O<sub>2</sub>) promoted AD-MSC proliferation, migration, and chondrogenic differentiation. | [83] |
|              | 2021 | Govoni et al. | Adv Med Sci | Severe hypoxic preconditioning (0.5% O<sub>2</sub>) promoted early chondrogenesis in AD-MSCs. | [85] |
| NP-MSCs      | 2013 | Li et al. | Cells Tissues Organs | Hypoxia (2% O<sub>2</sub>) inhibited the viability and proliferation of AD-MSCs and NP-MSCs but promoted their chondrogenic differentiation, and NP-MSCs exhibited higher biological activity than AD-MSCs. | [44] |
|              | 2021 | He et al. | Autophagy | Hypoxia (CoCl<sub>2</sub>) alleviated hydrostatic pressure-induced NP-MSC apoptosis. | [92] |
| UC-MSCs      | 2015 | Lee et al. | Stem Cells | Hypoxia (2.2% O<sub>2</sub>) promoted UC-MSC proliferation and migration compared with normoxia. | [93] |
| PMSCs        | 2012 | Yang et al. | The Spine Journal | | [94] |
promising therapeutic target for IDD therapy. In addition, other studies have also observed that hypoxia promotes the proliferation and migration of UC-MSCs [93]. The controversies about the effect of hypoxia on the viability and proliferation of AD-MSCs may be caused by the heterogeneity of different research protocols, medium components, oxygen tension and donors. Further studies are needed to explore and clarify.

In addition to the above types of MSCs, some researchers have also explored the effect of hypoxia on other rare types of MSCs. Some studies have shown that compared with normoxic environment, hypoxic environment promoted the early proliferation and differentiation of PMSCs into NP-like cells [94–97] and enhanced the migration ability of PMSCs by increasing the activity of matrix metalloproteinase-2 (MMP-2) [96]. Other studies have also observed the effect of hypoxia on AF-MSCs, SMSCs, and PB-MSCs and found that hypoxia enhanced the proliferation potential and stemness characteristics of AF-MSCs [98, 99] and PB-MSCs [100] and promoted the chondrogenic differentiation of SMSCs [101]. These results suggest that hypoxic culture conditions may serve as an effective strategy to maintain the function of MSCs (Table 2).

### 3.2. Nutrient Deficiency

IVD cells are supplied with essential nutrients by the diffusion from the blood supply through mainly the CEPs and disc tissue, and one of the key nutrients is glucose. A normal IVD is in an IVD-like low-glucose environment, with glucose concentrations dropping from about 5 mM in the outer AF to about 1 mM in the central NP [102]. After IDD, the glucose concentration in the NP is further decreased due to various factors including calcification of the CEPs and reduced blood supply to the vertebral body. Glucose concentrations in human degenerated NP average 0.603 ± 0.108 mM [71], and 0.5 mM is the minimum glucose level required for IVD cell survival [103]. The researchers explored the effect of glucose concentration on the biological behavior of BMSCs [104] and AD-MSCs [105]. They found that IVD-like low glucose (5.5 mM), although mildly inhibiting the viability of MSCs, promoted aggrecan expression and resulted in a small increase in proliferation compared to standard conditions (25 mM) [104, 105]. Some researchers also observed that the limited glucose condition inhibited the viability of NP-MSCs [106]. Hypoxia and nutritional deficiency often coexist in degenerated discs. Naqvi and Buckley [108] investigated the effect of different glucose concentrations on BMSCs under 5% hypoxia and found that IVD-like low glucose (5 mM) increased the accumulation of sulphated glycosaminoglycans (sGAG) and collagen. In contrast, too low glucose concentration (1 mM) promoted BMSC death and inhibited proliferation and the accumulation of sGAG and collagen [108]. These results suggest that IVD-like low-glucose conditions may be a positive factor for MSC transplantation in the treatment of IDD, while too low concentrations of glucose can have significant negative effects. Recently, Yang et al. [109] further studied and found that oxygen-glucose deprivation

| Cell sources | Year | Team | Journal | Results | Reference |
|--------------|------|------|---------|---------|-----------|
| AF-MSCs      | 2017 | Kwon et al. | Tissue Eng Regen Med | Hypoxia (5% O₂) promoted AF-MSC proliferation and stemness maintenance compared with normoxia. | [98] |
|              | 2020 | Casciaro et al. | Mech Ageing Dev | Hypoxia (1% O₂) promoted AF-MSC proliferation and stemness maintenance compared with normoxia. | [99] |
| SMSCs        | 2020 | Silva et al. | Glycoconj J | Hypoxia (5% O₂) promoted chondrogenic differentiation of SMSCs compared with normoxia. | [101] |
| PB-MSCs      | 2022 | Wang et al. | Front Endocrinol | Hypoxia (5% O₂) enhanced the proliferation, stemness, and multidirectional differentiation potential of PBSMCs compared with normoxia | [100] |

AD-MSCs: adipose-derived mesenchymal stem cells; AF-MSCs: amniotic fluid-derived mesenchymal stem cells; BMSCs: bone marrow-derived mesenchymal stem cells; ECM: extracellular matrix; IL-1β: interleukin-1β; IVD: intervertebral disc; NP: nucleus pulposus; NP-MSCs: nucleus pulposus-derived mesenchymal stem cells; PB-MSCs: peripheral blood-derived mesenchymal stem cells; PMSCs: placenta-derived mesenchymal stem cells; SMSCs: synovial-derived mesenchymal stem cells; TGF: transforming growth factor; UC-MSCs: umbilical cord-derived mesenchymal stem cells.
not only significantly inhibited the viability and adipogenesis of AD-MSCs but also inhibited cell proliferation and migration. Inhibition of receptor-interacting protein kinase-3 (RIP3) increased the viability, proliferation, and migration of AD-MSCs under oxygen-glucose deprivation and reduced unstable neovascularization and inhibited inflammatory responses [109].

In addition to glucose, serum is also an important nutrient for IVD cells. Parker et al. [110] found that both very low-serum and serum-free conditions maintained the chondrogenic differentiation ability of AD-MSCs. Since this study was conducted under normoxic conditions, Wan Safwani et al. [111] conducted a more in-depth study to clarify the exact effects of serum deprivation and hypoxia on MSCs. They found that serum deprivation inhibited the viability, proliferation, and adipogenic potential of AD-MSCs regardless of hypoxia but increased aggrecan gene expression levels, which in turn enhanced the chondrogenic differentiation of AD-MSCs [111]. Takahashi et al. [89] found that although serum deprivation inhibited the viability of MSCs, AD-MSCs were more tolerant to hypoxia, oxidative stress, and serum-free condition than BMSCs. In addition, hypoxia [112, 113] or glucose deprivation [114–116] under serum deprivation conditions can significantly reduce the viability and proliferation of MSCs and increase the production of reactive oxygen species and apoptosis. Berberine protects MSCs from autophagy and apoptosis through the AMP-activated protein kinase (AMPK) signaling pathway [113, 114]. Similarly, an appropriate concentration of acetyl-L-carnitine (ALC) can protect MSCs from nutrient deprivation-induced injury by enhancing the expression of survival signals and reducing the expression of death signals [115, 116]. Some studies have reported the impact of hypoxia and serum deprivation on rare type MSCs. Huang et al. [117, 118] found that hypoxia significantly enhanced the proliferation of PMSCs, while serum deprivation inhibited the growth of PMSCs. In addition, hypoxia and serum deprivation did not induce apoptosis of PMSCs, which may be related to the high expression of BCL-2 in serum deprivation [117]. These results indicate that PMSCs may be promising seed cells for ischemia-related tissue engineering.

Researchers usually use oxygen, glucose, and serum deprivation (OGD) conditions to simulate the in vivo ischemic microenvironment, and extensive studies have been conducted to enhance the adaptation of MSCs to the ischemic microenvironment. OGD conditions were shown to induce apoptosis in BMSCs, while extendin-4 [119] and icarin [120] could protect BMSCs from OGD-induced apoptosis by attenuating the ER stress signaling pathway. The viability and adipogenic differentiation of AD-MSCs were also shown to be inhibited by OGD conditions, while supplementation with exogenous transforming growth factor- (TGF-) β3 [121] or AMPK [122] could protect AD-MSC survival and adipogenesis. Tian et al. [123] conducted the first experiment to simulate the effects of complex OGD conditions on NP-MSCs. They found that hypoxia combined with nutrient deprivation inhibited the proliferation and induced apoptosis of NP-MSCs, enhanced the activity of caspase 3, and also inhibited the expression of functional genes (proteoglycan, collagen-I, and collagen-II) and stem cell-related genes (Oct4, Nanog, Jagged, and Notch1), and activation of phosphatidylinositol 3-kinase (PI3K) by insulin-like growth factor-1 (IGF-1) attenuated this effect [123]. These findings provide a feasible method for improving the therapeutic effect of MSC transplantation (Table 3).
study demonstrated that the acidic microenvironment of degenerating IVD can induce apoptosis of BMSCs by activating Ca^{2+}-permeable ASIC1a [132]. Recently, a team [133] found that TGF-β3 can be used to prime BMSCs to maintain cell survival in an acidic environment and promote the accumulation of NP-like matrix, thereby assisting BMSCs to overcome the harsh microenvironment of IVDs. In addition to BMSCs, other groups have also conducted similar studies on NP-MSCs. The researchers found that acidic conditions can reduce the viability, proliferation, and expression of aggrecan and collagen-I but had no effect on their viability.

### Table 3: Effects of nutrient deficiency on the biological behavior of MSCs.

| Cell sources | Year | Team | Journal | Results | Reference |
|--------------|------|------|---------|---------|-----------|
| BMSCs        | 2007 | Potier et al. | Tissue Eng | Serum deprivation (1% and 0% FBS) combined with hypoxia inhibited BMSC viability. | [112] |
|              | 2008 | Wuertz et al. | Spine | IVD-like glucose (1.0 mg/mL) promoted BMSC proliferation and expression of aggrecan and collagen-I but had no effect on their viability. | [104] |
|              | 2015 | Farrell et al. | Osteoarthritis Cartilage | Glucose deprivation (1.0 g/L) inhibited BMSC viability. | [107] |
|              | 2015 | Naqvi and Buckley | J Anat | Under hypoxic conditions (5% O_2), IVD-like glucose (5 mM) promoted the accumulation of sGAG and collagen compared with standard conditions (25 mM), whereas low glucose (1 mM) inhibited BMSC viability, proliferation, and accumulation of sGAG and collagen. | [108] |
|              | 2016 | He et al. | Int J Mol Med | Oxygen, glucose, and serum deprivation inhibited BMSC viability. | [119] |
| BMSCs        | 2020 | Liu et al. | Life Sci | Oxygen, glucose, and serum deprivation inhibited BMSC viability and proliferation. | [120] |
| AD-MSCs      | 2007 | Parker et al. | Cytotherapy | Both low-serum (0.5%) and serum-free maintained the chondrogenic differentiation ability of AD-MSCs. | [110] |
|              | 2012 | Liang et al. | J Transl Med | IVD-like glucose (1.0 mg/mL) slightly inhibited AD-MSC viability but increased aggrecan expression. | [105] |
|              | 2016 | Safwani et al. | Cytotherapy | Serum-free inhibited AD-MSC viability and proliferation but promoted their chondrogenic differentiation. | [111] |
|              | 2018 | Takahashi et al. | Cell Transplant | Serum-free conditions inhibited the viability of AD-MSCs and BMSCs, and AD-MSCs exhibited a higher survival rate than BMSCs. | [89] |
|              | 2018 | Ghorbani et al. | Nat Prod Res | Glucose-serum deprivation inhibited AD-MSC viability. | [114] |
| AD-MSCs      | 2018 | Wu et al. | Exp Ther Med | Oxygen, glucose, and serum deprivation inhibited AD-MSC viability. | [121] |
|              | 2018 | Li et al. | Acta Mol Cell Biol Lipids | Oxygen, glucose, and serum deprivation inhibited AD-MSC viability. | [122] |
| AD-MSCs      | 2020 | Abdolmaleki et al. | Cell Tissue Bank | Glucose-serum deprivation inhibited AD-MSC viability and proliferation. | [115] |
|              | 2021 | Pang et al. | Drug Des Devel Ther | Serum deprivation combined with hypoxia inhibited AD-MSC viability. | [113] |
|              | 2022 | Pan et al. | Cytotechnology | Serum deprivation inhibited AD-MSC viability, proliferation, and expression of stemness genes (Oct4, Nanog, and Sox-2). | [116] |
| NP-MSCs      | 2009 | Jünger et al. | Spine | Limited glucose (2.0 mg/mL) inhibited NP-MSC viability compared with sufficient glucose (4.5 mg/mL). | [106] |
|              | 2020 | Tian et al. | J Orthop Surg Res | Oxygen, glucose, and serum deprivation inhibited NP-MSC viability, proliferation, and expression of aggrecan, collagen-I, and collagen-II. | [123] |
| PMSCs        | 2009 | Huang et al. | Stem Cell Rev and Rep | Hypoxia (1% O_2) and serum deprivation did not induce apoptosis in PMSCs. | [117] |
|              | 2010 | Huang et al. | Cell biology international | Hypoxia (1% O2) promoted the proliferation of PMSCs, whereas serum deprivation inhibited the growth of PMSCs. | [118] |
| PMSCs        | 2009 | Huang et al. | Stem Cell Rev and Rep | Hypoxia (1% O_2) and serum deprivation did not induce apoptosis in PMSCs. | [117] |
|              | 2010 | Huang et al. | Cell biology international | Hypoxia (1% O2) promoted the proliferation of PMSCs, whereas serum deprivation inhibited the growth of PMSCs. | [118] |

AD-MSCs: adipose-derived mesenchymal stem cells; BMSCs: bone marrow-derived mesenchymal stem cells; FBS: fetal bovine serum; IVD: intervertebral disc; NP-MSCs: nucleus pulposus-derived mesenchymal stem cells; PMSCs: placenta-derived mesenchymal stem cells; sGAG: sulphated glycosaminoglycans.
ECM synthesis, and stem cell-related gene expression of NP-MSCs but increase the expression of ASIC1 and ASIC3 [40,134]. Sa12b, a wasp peptide, was found to reduce Ca\(^{2+}\) influx by inhibiting ASICs and Notch signaling pathways, thereby enhancing the biological activity of NP-MSCs in severely acidic environments [135]. Sa12b showed significant therapeutic potential in delaying IDD and improving LBP, providing a new perspective for the biological treatment of IDD [135] (Table 4).

### 3.4. Osmotic Cell Pressure

The aggrecan of the ECM is negatively charged under physiological conditions, and these negative charges explain the hyperosmolarity of the intervertebral disc [136,137]. The extracellular osmotic pressure of normal IVDs ranges from 43 to -496 mOsm/L, which is significantly higher than that of other tissues. When the IVD degenerates, aggrecan is gradually lost, and the extracellular osmotic pressure of the IVD gradually decreases, and in severe cases, it can drop to about 300 mOsm [138]. Wuertz et al. [104] and Liang et al. [105] found that IVD-like hyperosmolarity (485 mOsm) significantly inhibited the viability, proliferation, and expression of aggrecan and collagen-I of BMSCs and AD-MSCs compared with standard conditions (280 mOsm). Furthermore, under combined conditions, osmolarity and pH determine the effect of glucose [104,105]. Tao et al. [139] observed that IVD-like hyperosmolarity (400 mOsm) also had similar inhibitory effects on NP-MSC viability, proliferation, and ECM protein synthesis, although NP-MSCs showed a certain tolerance to hyperosmolarity (400 mOsm). A team has reported the effect of a wider range of cartilage tissue-specific osmolarity (400-600 mOsm) on ADMSCs. Increased osmolarity (400-600 mOsm) inhibited the viability, proliferation, and chondrogenic differentiation potential of AD-MSCs in a dose- and time-dependent manner compared to 300 mOsm and resulted in a spherical appearance of the cells [140]. In addition, the viability of AD-MSCs was strongly dependent on the type of culture, with AD-MSCs in monolayer culture being more tolerant to increased osmolarity compared to AD-MSCs in suspension, alginate-agarose hydrogel, and pellet cultures, thus emphasizing the importance of choosing relevant in vitro conditions according to the specifics of clinical application [140].

However, some researchers have observed different results regarding the effect of hyperosmolarity on the chondrogenic differentiation of MSCs. Caron et al. [141] reported the effect of hyperosmolarity using NaCl as an osmolyte on the proliferation and chondrogenic differentiation of two chondrogenic progenitor cells (ATDC5 and BMSCs). During chondrogenic differentiation of ATDC5 and BMSCs, hyperosmolarity of +100 mOsm increased the expression of chondrogenic markers (collagen-II, collagen-X, and aggrecan), while hyperosmolarity of +200 mOsm inhibited ATDC5 proliferation and chondrogenesis compared with control levels (285-310 mOsm) [141]. This result suggested that the optimal osmolarity for chondrogenic differentiation of ATDC5 and BMSCs was about 400 mOsm. In addition, Caron et al. [141] pointed out that the nuclear factor of activated T-cell 5 (Nfat5, also known as TonEBP) is involved in regulating the expression of chondrogenic genes in progenitor cells under normal and hypertonic conditions by transcriptionally affecting the

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**Table 4: Effects of pH on the biological behavior of MSCs.**

| Cell sources | Year | Team | Journal | Results | Reference |
|--------------|------|------|---------|---------|-----------|
| BMSCs        | 2008 | Wuertz et al. | Spine | IVD-like acidity (pH 6.8) inhibited BMSC viability, proliferation, and expression of aggrecan and collagen-I. | [104] |
| BMSCs        | 2009 | Wuertz et al. | Biochem Biophys Res Commun | Increased acidity inhibited BMSC viability, proliferation, and expression of aggrecan and collagen-I. | [124] |
| BMSCs        | 2016 | Naqvi and Buckley | Spine | Acidity with pH below 6.8 inhibited BMSC viability, proliferation, and accumulation of sGAG and collagen. | [127] |
| BMSCs        | 2019 | Cai et al. | Biosci Rep | Degenerated IVD-like acidity inhibited BMSC viability and proliferation. | [132] |
| BMSCs        | 2021 | Gansau et al. | Eur Cell Mater | Low pH (pH 6.8 and pH 6.5) inhibited AD-MSC viability and accumulation of sGAG and collagen compared with standard pH (pH 7.4). | [133] |
| AD-MSCs      | 2012 | Li et al. | J Transl Med | IVD-like acidity (pH 6.8) inhibited AD-MSC viability, proliferation, and expression of aggrecan and collagen-I. | [105] |
| AD-MSCs      | 2014 | Han et al. | Cells Tissues Organs | Acidic pH inhibited AD-MSC viability, proliferation, and expression of aggrecan, collagen-I and collagen-II. | [125] |
| NP-MSCs      | 2017 | Liu et al. | Stem Cells Dev. Aging | Acidic pH inhibited NP-MSC viability, proliferation, and ECM synthesis. | [40] |
| NP-MSCs      | 2021 | Ding et al. | Front Bioeng Biotechnol | Acidic conditions (pH 6.2) inhibited NP-MSC viability, proliferation, and ECM synthesis. | [134] |
| NP-MSCs      | 2022 | Wang et al. | | | |
Table 5: Effects of osmotic cell pressure on the biological behavior of MSCs.

| Cell sources | Year | Team                      | Journal            | Results                                                                 | Reference |
|--------------|------|---------------------------|--------------------|----------------------------------------------------------------------|----------|
| BMSCs        | 2008 | Wuertz et al.             | Spine              | IVD-like osmolarity (485 mOsm) inhibited BMSC viability, proliferation, and expression of aggrecan and collagen-I compared with controls (280 mOsm). | [104]    |
| AD-MSCs      | 2012 | Liang et al.              | J Transl Med       | The increased osmolarity (380 mOsm) promoted the chondrogenic differentiation of BMSCs compared with controls (285 ± 5 mOsm). | [141]    |
|              | 2016 | Potočar et al.            | PLos One.          | IVD-like osmolarity (485 mOsm) inhibited AD-MSC viability, proliferation, and expression of aggrecan and collagen-I compared with controls (280 mOsm). | [105]    |
|              | 2018 | Ahmadyan et al.           | Appl Biochem       | All hyperosmolar conditions (350, 450, and 550 mOsm) promoted the chondrogenic differentiation of AD-MSCs, and only NaCl 550 mOsm inhibited AD-MSC proliferation. | [142]    |
|              | 2018 | Ahmadyan et al.           | Cell Mol Biol      | Hyperosmolar conditions (450 mOsm) promoted the chondrogenic differentiation of AD-MSCs compared with controls (350 mOsm). | [143]    |
|              | 2020 | Zhang et al.              | Mol Cell Biochem   | Compared with controls (300 mOsm), 400 mOsm osmolarity promoted NP-like differentiation of AD-MSCs, whereas 500 mOsm osmolarity inhibited NP-like differentiation of AD-MSCs and increased osmolarity (400 and 500 mOsm) inhibited their viability and proliferation. | [144]    |
|              | 2021 | Alinezhad-Bermi et al.    | In Vitro Cell Dev Biol Anim | Certain dose of hyperosmolarity (480 mOsm) promoted the chondrogenic differentiation of AD-MSCs compared with controls (350 mOsm). | [145]    |
| NP-MSCs      | 2013 | Tao et al.                | Cell Biol Int      | IVD-like osmolarity (400 mOsm) slightly inhibited NP-MSC viability and significantly inhibited their proliferation and expression of sox-9, aggrecan, and collagen-II compared with controls (280 mOsm). | [139]    |
|              | 2018 | Li et al.                 | Cells Tissues Organs | Compared with controls (300 mOsm), the hyperosmolarity (430 and 500 mOsm) of healthy IVD inhibited the proliferation and chondrogenic differentiation of NP-MSCs, whereas the relative hyperosmolarity (400 mOsm) promoted their proliferation and chondrogenic differentiation. | [146]    |

AD-MSCs: adipose-derived mesenchymal stem cells; BMSCs: bone-marrow mesenchymal stem cells; IVD: intervertebral disc; NP: nucleus pulposus; NP-MSCs: nucleus pulposus-derived mesenchymal stem cells.

ey early expression of sox-9 (SRY- (sex-determining region Y-) box 9). Similar results were also observed in related studies of AD-MSCs. Ahmadyan et al. [142] assessed the expression of chondrogenic and hypertrophic markers and vascular endothelial growth factor (VEGF) secretion in AD-MSCs at three osmolarity levels using three different osmolytes, NaCl, sorbitol, and polyethylene glycol (PEG). As expected, all hyperosmolar conditions led to enhanced chondrogenesis with slightly varying degrees, and hypertonic conditions positively correlated with early expression of specific chondrocyte markers [142]. Nfat5 was observed to be involved in osmoadaptation of all treatments in varying degrees [142]. PEG and sorbitol have higher cartilage-promoting and hypertrophy-inhibiting effects compared to NaCl, which exacerbates hypertrophy [142, 143]. In this study, the inhibitory effect on AD-MSC proliferation was only observed in NaCl 550 mOsm, and the other treatments showed no inhibitory effect on cell proliferation [142]. These findings are basically consistent with the findings of Caron et al. [141].

Although it has been widely reported that hyperosmolarity promotes chondrogenic differentiation of MSCs, unlike Ahmadyan et al. [142] who reported that hyperosmolarity was positively correlated with the early expression of specific chondrocyte markers, the researchers found that higher or lower osmolarity may negatively affect chondrogenic differentiation. Zhang et al. [144] observed that high osmolarity (400 mOsm and 500 mOsm) inhibited the viability and proliferation of AD-MSCs compared with 300 mOsm, which was the same as the previous study. Interestingly, a slight increase in osmolarity to near physiological levels (400 mOsm) induced AD-MSCs to differentiate into an NP-like phenotype. Lower (300 mOsm) or higher (500 mOsm) osmolarity was found to decrease the expression of NP-like markers and ECM synthesis, suggesting a potential optimal osmotic window for successful differentiation of AD-MSCs [144]. In addition, it was reported for the first time that histone demethylase KDM4B induced NP-like differentiation of AD-MSCs through H3K9me3/2 targeting Foxa1/2 under hypertonic conditions [144]. Some researchers have observed that 480 mOsm hyperosmolarity can improve advanced cartilage formation by upregulating the expression of cartilage-specific markers and reducing mineralization rate and angiogenic potential [145]. Li et al. [146] cultured NP-MSCs in media at 300, 400, 430, and 500 mOsm, mimicking the osmotic pressures of serious degenerative, moderately degenerative, and healthy IVD. The results showed that, compared to 300 mOsm, hyperosmolarity...
of healthy IVD (430 and 500 mOsm) inhibited the proliferation and chondrogenic differentiation of NP-MSCs. The relative hypoosmotic condition of moderately degenerative IVD (400 mOsm) led to great proliferation and chondrogenic differentiation capacity [146]. These results suggest that the relatively hypotonic condition prevalent in degenerative IVD may provide a more permissive environment for chondrogenic differentiation of MSCs (Table 5).

3.5. Mechanical Stress. In daily life, the IVD tissue is stimulated by various mechanical loads such as compression, flexion and extension, hydrostatic pressure, torsion, and shear forces. Different mechanical loading intensities, durations, frequencies, and directions significantly affect the metabolism of IVD cells and ECM [147]. Static loading is thought to inhibit nutrient transport and necessary gas exchange and is associated with increased disc cell death and ECM catabolism, whereas dynamic loading is thought to promote macromolecular nutrient transport and is associated with increased ECM anabolism [148, 149]. These mechanical loads have an effect not only on the disc tissue but also on the biological behavior of the implanted MSCs. Previous studies have demonstrated that cyclic compressive loading can promote chondrogenic differentiation of BMSCs by upregulating TGF-β gene expression and protein synthesis [150–153]. These findings suggest that cyclic mechanical loading can be exploited to promote chondrogenic differentiation of the implanted BMSCs. Later, researchers conducted more in-depth studies to optimize the amplitude of cyclic compression that stimulates ECM synthesis. They found that low-amplitude dynamic compressive loading (5% amplitude) promoted ECM synthesis by inhibiting the transient receptor potential vanilloid 4 (TRPV4) without affecting the viability of AD-MSCs, whereas high-amplitude dynamic compressive loading inhibited their viability and ECM synthesis compared with the no-compression loading group [154]. Cyclic compression loading was also found to stimulate the proliferation and chondrogenic differentiation of AD-MSCs, and dynamic compression combined with exogenous sox-9 [155] showed additive effects on the chondrogenic differentiation of AD-MSCs. Although dynamic compressive loading promoted the proliferation and chondrogenic differentiation of MSCs, static compressive loading negatively affected the biological behavior of MSCs. Liang et al. [156] studied the effects of static compressive loading on the biological behavior of NP-MSCs. They found that static compressive loading significantly inhibited the viability, migration, colony-forming ability, and multiple differentiation potentials of NP-MSCs and reduced the stemness of NP-MSCs, which may be one of the mechanisms for the failure of endogenous repair of IDD [156].

Since the IVD tissue is usually not affected by a single type of mechanical load under physiological conditions, in order to study the effect of mechanical load on complex motion, some researchers established a fibrin-polyurethane scaffold compound culture system to analyze the effect of cyclic compressive load combined with surface shear stress on BMSCs [157, 158]. They found that the chondrogenic differentiation of BMSCs was affected by the frequency and amplitude of dynamic compression and surface shear stress, and the chondrogenic differentiation potential of BMSCs could be further enhanced by changing the frequency and compression amplitude [157, 158]. Similar to cyclic compressive loading, cyclic hydrostatic pressure was also shown to promote chondrogenic differentiation of BMSCs [159, 160] and AD-MSCs [161] in a dose- and time-dependent manner. Dai et al. [162] found that dynamic compression of dynamic hydrostatic pressure not only promoted the proliferation and differentiation of AD-MSCs into NP-like manifestations but also showed a cumulative effect on NP-like differentiation in combination with coculture with nucleus pulposus cells. To determine whether hydrostatic pressure and platelet-rich fibrin activate canonical or noncanonical Wnt signaling in BMSCs, BMSCs cocultured with platelet-rich fibrin (PRF) were hydrostatically loaded. [163] conducted an experiment to explore the biosignaling mechanism of BMSCs stimulated by hydrostatic pressure. They found that 120 kPa hydrostatic pressure activates both Wnt/β-catenin signaling and Wnt/Ca²⁺ signaling, and growth factors released by PRF may reverse the stress-promoting effect of Wnt/Ca²⁺ signaling [163]. This result indicated that Wnt signaling was involved in stress-promoting chondrogenesis in BMSCs cocultured with PRF, and the canonical and noncanonical pathways played different roles in this process. Some researchers have observed that intermittent compression of excessive hydrostatic pressure (1.0 MPa) induces apoptosis of NP-MSCs, and hypoxia can alleviate this negative effect [92].

In addition to compressive loads, shear forces, and hydrostatic pressure, the intervertebral disc tissue is also subjected to tensile loads, which also affect the biological behavior of the implanted MSCs. Connelly et al. [164] studied the effect of cyclic tensile loading (10%, 1 Hz) on the chondrogenic differentiation of BMSCs and found that cyclic tensile loading specifically stimulated the synthesis of collagen-I but had no effect on the synthesis of collagen-II, aggregan, or osteocalcin. Overall, this study demonstrated that cyclic tensile loading promoted the fibrochondrocyte-like differentiation of BMSCs, and Baker et al. [165] observed similar results in a subsequent study. Recently, Abusharkh et al. [166] found that coculture of AD-MSCs with articular chondrocytes (ACHs) under cyclic tensile stress of 10% and 1 Hz could promote the production of cartilage ECM of AD-MSCs, and 1:3 AChs:AD-MSCs was a good coculture ratio. The results of this study provide new ideas for optimizing tissue engineering for stem cell therapy (Table 6).

3.6. Inflammatory Cytokines and Proteinases. IDD is a complex process. Activation of autoimmune responses is an important pathological mechanism leading to IDD, and inflammatory mediators may be a key factor in its occurrence and progression. Under physiological conditions, the NP tissue is trapped by the surrounding AF and CEPs, and this unique structure isolates the NP from the immune system of the host, thereby maintaining the IVD immune privilege [167, 168]. When IDD occurs, the AF is damaged to produce fissures, and the exposed NP tissue activates the autoimmune response system, thereby stimulating
immunocyte activation and infiltration of inflammatory mediators. Meanwhile, the recruitment of immunocytes and the infiltration of inflammatory mediators in turn accelerate the progression of IDD [169, 170]. The autoimmune response to NP involves various types of immunocytes, including macrophages, T lymphocytes, and neutrophils, and macrophages may play a significant role in this pathological process [169]. In IDD patients, the expression levels of various proinflammatory cytokines are upregulated. Common highly expressed proinflammatory cytokines include tumor necrosis factor-α (TNF-α), interleukin-1α/β (IL-1α/β), IL-6, IL-17, IL-8, interferon-γ (IFN-γ), prostaglandin E2 (PGE2), and substance P, among which TNF-α, IL-1β, IL-6, and IL-17 are the key inflammatory mediators [171–173]. By activating the nuclear factor kappa-B (NF-κB), mitogen-activated protein kinase (MAPK), and Toll-like receptor (TLR) signaling pathways, these proinflammatory cytokines can induce disc degeneration by promoting catabolic enzymes, such as a disintegrin and metalloproteinase with thrombospondin motif- (ADAMTS-) 4 and -5 and MMP-1,

| Year | Team | Journal | Results | Reference |
|------|------|---------|---------|-----------|
| 2003 | Angele et al. | J Orthop Res | Cyclic hydrostatic pressure promoted chondrogenic differentiation of BMSCs. | [159] |
| 2004 | Angele et al. | Biorheology | Cyclic compressive loading promoted chondrogenic differentiation of BMSCs. | [150] |
| 2004 | Huang et al. | Stem Cells | Cyclic compressive loading (1 Hz, 10% magnitude) promoted chondrogenic differentiation of BMSCs. | [152] |
| 2005 | Huang et al. | Stem Cells | Cyclic compressive loading (1 Hz, 15% magnitude) promoted chondrogenic differentiation of BMSCs. | [151] |
| 2006 | Miyanishi et al. | Tissue Eng | Different levels of cyclic hydrostatic pressure (1 Hz, 0.1, 1, and 10 MPa) differentially regulated BMSC chondrogenesis in the presence of TGF-β3. | [160] |
| 2007 | Mow et al. | Stem Cells | Cyclic compressive loading (1 Hz, 10% ± 3% magnitude) promoted the chondrogenic differentiation of BMSCs. | [153] |
| 2010 | Li et al. | Tissue Eng Part A | Cyclic compressive loading combined with surface shear force promoted the chondrogenic differentiation of BMSCs. | [157] |
| 2010 | Li et al. | J Cell Mol Med | Cyclic compressive loading combined with surface shear stress promoted the chondrogenic differentiation of BMSCs. | [158] |
| 2010 | Connelly et al. | Tissue Eng Part A | Cyclic tensile loading (1 Hz, 10% magnitude) specifically promoted the synthesis of collagen-I by BMSCs. | [164] |
| 2011 | Baker et al. | Tissue Eng Part A | Cyclic tensile loading (1 Hz, 3% magnitude) promoted ECM synthesis by BMSCs. | [165] |
| 2018 | Gan et al. | Stem Cells Int | Compared with no-compression controls, low-magnitude dynamic compressive loading (5%, 1 Hz) promoted ECM synthesis without affecting BMSC viability, whereas high-magnitude dynamic compressive loading (10% and 20%, 1 Hz) inhibited their viability and ECM synthesis. | [154] |
| 2022 | Cheng et al. | Tissue Eng Regen Med | Hydrostatic pressure promoted chondrogenic differentiation of BMSCs cocultured with RPF. | [163] |
| 2009 | Ogawa et al. | Tissue Eng Part A | Cyclic hydrostatic pressure (0.5 Hz, 0-0.5 MPa) promoted the chondrogenic differentiation of AD-MSCs. | [161] |
| 2014 | Dai et al. | J Biomech | Dynamic compression of intermittent dynamic hydrostatic pressure promoted the proliferation of AD-MSCs and induces their differentiation into NP-like cells. | [162] |
| 2015 | Zhang et al. | Eur Rev Med Pharmacol Sci | Cyclic compressive loading combined with exogenous sox-9 promoted the proliferation and chondrogenic differentiation of AD-MSCs. | [155] |
| 2021 | Abusharkh et al. | In Vitro Cell Dev Biol Anim | High cyclic tensile loading (10% magnitude) promoted ECM synthesis without affecting the viability of AD-MSCs. | [166] |
| 2018 | Liang et al. | Stem Cells Int | Compressive loading (1 MPa) inhibited NP-MSC viability, migration, and expression of stemness genes (Sox-2 and Oct4). | [156] |
| 2021 | He et al. | Autophagy | Hydrostatic pressure (1.0 MPa) inhibited NP-MSC viability. | [92] |

AD-MSCs: adipose-derived mesenchymal stem cells; BMSCs: bone marrow-derived mesenchymal stem cells; ECM: extracellular matrix; TGF-β3: transforming growth factor-β3; NP: nucleus pulposus; NP-MSCs: nucleus pulposus-derived mesenchymal stem cells.
-2, -3, -4, -13, and -14, and decreasing anabolic ECM proteins, such as aggrecan and collagen-II [174, 175]. In addition to proinflammatory cytokines, anti-inflammatory cytokines, such as TGF-β, growth, and differentiation factor 5 (GDF-5), GDF-6, IL-4, and IL-10, can also be observed in IDD patients, which can promote IVD repair and relieve pain symptoms to a certain extent [173, 176]. The paradoxical effects of the expression of these inflammatory mediators are closely related to macrophage polarization and the distribution of different types of macrophages [177, 178].

The expression of inflammatory mediators can not only affect the degeneration process of the IVD but also affect the biological behavior of the implanted MSCs. Felka et al. [77] found that supplementation of IL-1β had a negative effect on the chondrogenic differentiation potential of MSCs, while hypoxia reduced the inhibitory effect of IL-1β on the chondrogenic differentiation of BMSCs. Subsequently, some researchers found that IL-1β could induce MSC migration, adhesion, and leukocyte chemotaxis by activating the NF-κB signaling pathway but did not affect the viability and proliferation of BMSCs [179, 180]. TNF-α has also been observed to enhance the migratory capacity of BMSCs through the NF-κB signaling pathway [181–183]. Interestingly, similar results were not observed for all types of MSCs. Brandt et al. [184] studied the effect of different inflammatory conditions on the functional properties of AD-MSCs. They found that high concentrations of proinflammatory cytokines (IL-1β: 10 ng/mL and/or TNF-α: 50 ng/mL) promoted the proliferation and osteogenic differentiation of AD-MSCs but decreased cell viability and chondrogenic and adipogenic differentiation potential. In contrast, Mohammadpour et al. [185] pointed out that TNF-α alone had no effect on the proliferation of AD-MSCs, but combined with IFN-γ could significantly promote the proliferation of AD-MSCs. Cheng et al. [186] investigated the regulatory effects of TNF-α at high and low concentrations on the biological behaviors of healthy rat NP-MSCs. It was found that a high concentration of TNF-α (50–200 ng/mL) could induce apoptosis of NP-MSCs, whereas a relatively low TNF-α concentration (0.1–10 ng/mL) promoted the proliferation and migration of NP-MSCs but inhibited their differentiation toward NP-like cells [186]. Moreover, the NF-κB signaling pathway was activated during the differentiation of NP-MSCs inhibited by TNF-α [186]. Another team [187] exposed UC-MSCs to a proinflammatory cytokine environment in vivo and observed that IL-1β and TNF-α, although inhibited the proliferation and adipogenesis of UC-MSCs, enhanced their chondrogenic differentiation capacity. The differences in these findings may be related to the different types of MSCs, culture conditions, and concentrations of proinflammatory cytokines.

As one of the important proinflammatory cytokines in IDD, the effects of IL-6 and IL-17 on the biological behavior of MSCs have also been investigated. Studies have shown that IL-6 inhibits the chondrogenic differentiation of BMSCs, and the JAK/STAT pathway is activated during this process [187, 188]. In addition, IL-6 was also found to limit BMSC proliferation but improve their migration [189]. IL-17 is a proinflammatory cytokine mainly secreted by Th17 cells. Huang et al. [190] reported that IL-17 could directly stimulate BMSC proliferation, migration, and osteoblast differentiation, and these response mechanisms were shown to involve the production of reactive oxygen species. Mojsilović et al. [191] also observed the promoting effect of IL-17 on the proliferation of BMSCs and pointed out that mitogen-activated protein kinase (MAPK) signaling pathways mediated by p38 and extracellular signal-regulated kinase (ERK) were involved in this process. Recently, a team found that IL-17 pretreatment can be used to enhance the homing ability and immunosuppressive function of BMSCs, thereby achieving the purpose of prolonging graft survival and reducing graft rejection. This finding provides a novel and useful research focus for developing MSCs for transplantation techniques [192].

Not only proinflammatory cytokines but also anti-inflammatory cytokines in IDD can regulate the biological behaviors of engrafted MSCs. In mammals, the TGF-β family includes three TGF-β members, TGF-β1, TGF-β2, and TGF-β3. TGF-β signaling plays a critical role in IVD development, growth, and tissue homeostasis, but overactivation of TGF-β signaling can lead to aggravation of IDD [193]. GDF-5 and GDF-6 are members of the TGF-β superfamily, which also play an important role in spine development. Studies have shown that TGF-β, GDF-5, and GDF-6 can all promote the differentiation of MSCs to an NP-like phenotype [80, 194–197]. And compared with TGF-β or GDF-5, GDF-6 can drive MSCs to produce more proteoglycan-rich ECM, which is more suitable for promoting the differentiation of MSCs to NP-like phenotype [196]. Furthermore, the effect is more pronounced in AD-MSCs than BMSCs [196]. Later, Li et al. [198] found that TGF-β3 mainly promotes the ingrowth of AD-MSCs into cartilage through the Wnt5a/β-catenin signaling pathway, which is expected to provide new ideas for the field of cartilage regeneration. IL-4 is another important anti-inflammatory cytokine in the body. Pretreatment with IL-4 can improve the migration ability of MSCs and the expression level of certain proteins, which has a beneficial effect on the regeneration process of MSCs [189, 199, 200].

In addition to common types of MSCs, researchers have also explored the effects of inflammatory cytokines on rare types of MSCs. Li et al. [201] found that proinflammatory cytokines (IL-1, IL-6, and IL-8) promoted the proliferation of PMSCs in a dose-dependent manner, peaking at concentrations of 10 ng/mL of IL-1 and IL-6 and 150 ng/mL of IL-8. In addition, anti-inflammatory cytokine (IL-4) inhibited the proliferation of PMSCs in a dose-dependent manner [201]. However, Zhang et al. [202] separately studied the interaction between IL-1β and PMSCs, and the results showed that IL-1β (20 ng/mL or 30 ng/mL) regulates programmed death ligand 1 (PD-L1) expression through JAK and NF-κB pathways, thereby inhibiting the adhesion and proliferation of PMSCs. IFN-γ was also found to inhibit the proliferation and migration of PMSCs [203]. Recently, a team [204] conducted a similar study using PB-MSCs, and they observed that compared with the unstimulated control group, IL-1β promoted cell migration, while TNF-α inhibited the migration of PB-MSCs. In order to compare the different responses of various types of MSCs to inflammatory
Table 7: Effects of inflammatory cytokines and proteases on the biological behavior of MSCs.

| Cell sources | Year | Team | Journal | Inflammatory cytokines | Results | Reference |
|--------------|------|------|---------|------------------------|---------|-----------|
| BMSCs        | 2007 | Ponte et al. | Stem Cells | TNF-α (1 ng/mL) promoted BMSC migration. | [181]   |
|              | 2009 | Felka et al. | Osteoarthritis Cartilage | IL-1β (2 ng/mL) inhibited the chondrogenic differentiation of BMSCs, while hypoxia (2% O2) reduced the inhibitory effect of IL-1β on the chondrogenic differentiation of BMSCs. | [77]    |
|              | 2009 | Huang et al. | Cell Death Differ | IL-17 (50 ng/mL) promoted BMSC proliferation and migration. | [190]   |
|              | 2011 | Mojsilović et al. | Cell Tissue Res | IL-17 (5-50 ng/mL) and bFGF (1 ng/mL) promoted the proliferation of BMSCs by activating p38 and ERK-mediated MAPK signaling pathway. | [191]   |
|              | 2011 | Gantenbein-Ritter et al. | Eur Spine J | TGF-β1 (10 ng/mL) and GDF-5 (100 ng/mL) promoted the differentiation of BMSCs into NP-like cells. | [194]   |
|              | 2017 | Wang et al. | Eur Cell Mater | Hypoxia (2% O2) and GDF5 (100 ng/mL) promoted the differentiation of BMSCs to a NP-like phenotype. | [80]    |
|              | 2018 | Stoyanov et al. | Stem Cell Res Ther | TNF-α promoted (30 ng/mL) BMSC migration. | [182]   |
|              | 2018 | Teixeira et al. | Spine | IL-1β (10 ng/mL) promoted BMSC migration but had no effect on cell viability. | [180]   |
|              | 2018 | Ma et al. | Ann Transplant | IL-17 (50 ng/mL) increased the homing and immunosuppressive abilities of BMSCs. | [192]   |
|              | 2019 | Kasprzycka et al. | Stem Cell Res Ther | IL-4 (50 ng/mL) and IL-6 (50 ng/mL) inhibit BMSC proliferation but promote their migration. | [189]   |
|              | 2021 | Xie et al. | Nat Commun | High concentration of TNF-α (100 ng/mL) promoted the directional migration of BMSCs. Compared with TGF-β (10 ng/mL) or GDF-5 (100 ng/mL), GDF-6 (100 ng/mL) was more suitable for promoting the differentiation of MSCs to NP-like phenotype, and it was more pronounced in AD-MSCs than in BMSCs. | [196]   |
| Inflammatory cytokines | | | | | |
| AD-MSCs      | 2016 | Colombier et al. | Stem Cells | TGF-β1 (10 ng/mL) combined with GDF-5 (100 ng/mL) promoted the chondrogenic differentiation of AD-MSCs. | [197]   |
|              | 2016 | Mohammadpour et al. | Immunopharmacology and Immunotoxicology | IFN-γ (10 ng/mL) alone or in combination with TNF-α (10 ng/mL) promoted the proliferation of AD-MSCs, while TNF-α (10 ng/mL) alone had no effect on the proliferation of AD-MSCs. | [185]   |
|              | 2018 | Brandt et al. | Int J Mol Sci. | High concentrations of proinflammatory cytokines (IL-1β: 10 ng/mL and/or TNF-α: 50 ng/mL) promoted the proliferation and osteogenic differentiation of AD-MSCs but inhibited cell viability and chondrogenic differentiation. | [184]   |
|              | 2020 | Li et al. | Biochem Biophys Res Commun. | TGF-β3 (100 ng/mL) promoted the chondrogenic differentiation of AD-MSCs. | [198]   |
|              | 2020 | Archacka et al. | Cells | IL-4 (10 ng/mL) enhanced the migration ability of AD-MSCs. | [199]   |
|              | 2020 | Zimowska et al. | Int J Mol Sci | IL-4 (10 ng/mL) promoted AD-MSC proliferation and migration. | [200]   |
|              | 2015 | Tao et al. | Growth Factors | TGF-β3 (10 ng/mL) combined with IGF-1 (10 ng/mL) promoted NP-MSC viability and differentiation towards NP-like phenotype. | [195]   |
| NP-MSCs      | 2019 | Cheng et al. | J Cell Biochem | High concentrations of TNF-α (50-200 ng/mL) induced apoptosis of NP-MSCs, whereas a relatively low concentrations of TNF-α (0.1-10 ng/mL) promoted NP-MSC proliferation and migration but inhibited their differentiation into NP-like cells. | [186]   |
| UC-MSCs      | 2018 | Yang et al. | Mol Cell Biochem | IL-1β (20 ng/mL) and TNF-α (20 ng/mL) inhibited the proliferation of UC-MSCs but promoted their chondrogenic differentiation, and IL-6 (20 ng/mL) inhibited the chondrogenic differentiation of UC-MSCs. | [187]   |
### Table 7: Continued.

| Cell sources | Year  | Team          | Journal                  | Results                                                                                                                                                                                                 | Reference |
|--------------|-------|---------------|--------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| PMSCs        | 2007  | Li et al.     | Cells Tissues Organs     | Proinflammatory cytokines (IL-1, IL-6, and IL-8) promoted the proliferation of PMSCs in a dose-dependent manner, while anti-inflammatory cytokine (IL-4) inhibited the proliferation of PMSCs in a dose-dependent manner. | [201]     |
| AMSCs        | 2018  | Yi et al.     | Cellular Immunology      | IFN-γ (20 ng/mL) inhibited the proliferation and migration of PMSCs.                                                                                                                                 | [203]     |
| AF-MSCs      | 2020  | Zhang et al.  | Cell Immunol             | IL-1β (20 ng/mL or 30 ng/mL) promoted the migration of PMSCs but inhibited cell proliferation.                                                                                                                                                                     | [202]     |
| AMSCs        | 2019  | Borem et al.  | J Orthop Res             | Compared with AD-MSCS, the same inflammation conditions promoted chondrogenic differentiation of AMSC.                                                                                                                                                     | [205]     |
| PB-MSCs      | 2018  | Calle et al.  | Stem Cell Res Ther       | IL-1β promoted the migration of AD-MSCs and PB-MSCs.                                                                                                                                                                                                         | [207]     |
| BMSCs        | 2006  | Neth et al.   | Stem Cells               | MMP-1-MMP promoted BMSC proliferation and migration.                                                                                                                                                                                                       | [210]     |
| MMP          | 2007  | Ries et al.   | Blood                    | MMP-2, MT1-MMP, and TIMP-2 promoted BMSC migration, while TIMP-1 inhibited their migration.                                                                                                                                                                    | [211]     |
| AD-MSCs      | 2010  | Lu et al.     | Blood                    | MT1-MMP dominated BMSC migration and differentiation into NP-like cells.                                                                                                                                                                                      | [212]     |
| MMP          | 2013  | Sun et al.    | Cell Signal              | MT1-MMP promoted BMSC proliferation.                                                                                                                                                                                                                       | [213]     |
| MMP          | 2016  | Gao et al.    | Mol Reprod Dev           | Silencing MMP-2 reduced BMSC proliferation and migration.                                                                                                                                                                                                     | [214]     |
| AD-MSCs      | 2019  | He et al.     | Am J Physiol Heart Circ Physiol | MMP-9 promoted AD-MSC proliferation and migration.                                                                                                                                                                                                             | [215]     |
| NP-MSCs      | 2020  | Rong et al.   | Dermatol Ther            | Overexpression of MMP-3 reduced the expression level of collagen-I in AD-MSCs.                                                                                                                                                                              | [216]     |
| UC-MSCs      | 2019  | Zhang et al.  | Cell Signal              | Downregulation of MMP-3 promoted the chondrogenic differentiation of NP-MSCs.                                                                                                                                                                               | [217]     |
| UC-MSCs      | 2014  | Marquez-Curtis et al. | Stem Cells Int                | Increased expression of MMP-2 promoted UC-MSC migration.                                                                                                                                                                                                       | [218]     |

AD-MSCs: adipose-derived mesenchymal stem cells; AF-MSCs: amniotic fluid-derived mesenchymal stem cells; AMSCs: amniotic membrane-derived mesenchymal stem cells; BMSCs: bone marrow-derived mesenchymal stem cells; GDF: growth and differentiation factor; IL: interleukin; IVD: intervertebral disc; MMP: matrix metalloproteinases; MT1-MMP: membrane-type matrix metalloproteinases; NP-MSCs: nucleus pulposus-derived mesenchymal stem cells; PB-MSCs: peripheral blood-derived mesenchymal stem cells; PMSCs: placenta-derived mesenchymal stem cells; TGF: transforming growth factor; TIMPs: tissue inhibitors of metalloproteinases; TNF-α: tumor necrosis factor-α; UC-MSCs: umbilical cord-derived mesenchymal stem cells.
cytokines, comparative studies between stem cells have also been carried out. Park et al. [205] evaluated the effect of anti-inflammatory cytokines (TGF-β3) on the differentiation of BMSCs, AD-MSCs, and AF-MSCs. Compared with the control group without TGF-β3, the chondrogenic differentiation potential of these three types of MSCs have been improved. Furthermore, the chondrogenic potential of AF-MSCs were relatively low compared to BMSCs and AD-MSCs [205]. In a previous study, Calle et al. [206] compared the effects of proinflammatory cytokines on the migration properties of MSCs and showed that IL-1β promoted the migration of AD-MSCs and PB-MSCs compared with the control group. Borem et al. [207] cultured human AD-MSCs and AMSCs in medium supplemented with IL-1β and TNF-α to directly compare the impact of IDD inflammation on their effector functions. Compared with noninflammatory controls, inflammatory cultures promoted the proliferation of MSCs but inhibited their chondrogenic differentiation, and these effects were more pronounced in AD-MSCs [207]. These findings may help researchers understand which source of MSCs may be the best choice for IVD regeneration.

MMPs are a family of neo-dependent proteolytic enzymes that play an important role in the catabolism of ECM. Their activity is modulated by specific inhibitors known as tissue inhibitors of metalloproteinases (TIMPs). Dysregulated expression and activity of MMPs is an important cause of ECM catabolism in IDD [208]. Increased expression of MMPs, including MMP-1, -2, -3, -7, -8, -9, -10, -13, and -14 and ADAMTS-4, has been repeatedly observed during IDD, some of which are related with the severity of degradation [21, 209]. MMPs can not only regulate the matrix metabolic activities of IVD tissues but also regulate the biological behavior of the implanted MSCs. Studies have found that membrane-type MMP-1 (MT1-MMP) promotes the proliferation and migration of BMSCs through the Wnt signaling pathway [210–213] and induces the differentiation of BMSCs into NP-like cells [212]. In addition, silencing MMP-2 also inhibited the proliferation and migration of MSCs [210, 214]. MMPs also affect the biological behavior of other types of MSCs. MMP-9 can promote the proliferation and migration of AD-MSCs [215]. Overexpression of MMP-3 decreased the expression level of collagen-I in AD-MSCs [216], and its downregulation promoted the chondrogenic differentiation of NP-MSCs [217]. The increased expression of MMP-2 can promote the proliferation and migration of UC-MSCs while maintaining their chondrogenic differentiation potential [218]. There are many types of MMPs, which play an important role in regulating the biological behavior of the implanted stem cells. However, there are still relatively few related studies, so it is necessary to carry out extensive related research in the follow-up (Table 7).

4. Conclusions

Although the biological behavior of the implanted MSCs will be affected by various IVD microenvironmental factors, they can also secrete some bioactive factors through the paracrine pathway to regulate the microenvironment in which they are located, so as to better adapt to the IVD microenvironment. With the deepening of the understanding of microenvironmental factors, more and more researchers have begun to use microenvironmental pretreatment or develop various scaffold materials to reduce the adverse effects of the microenvironment on implanted MSCs, so as to maximize the therapeutic effect of stem cells for repairing degenerated IVDs. The effect of IVD microenvironment on the biological behavior of MSCs is a complex process with multiple factors. Although the current understanding of it has greatly improved, the interaction of transplanted MSCs with the complex microenvironment of IVD and the exact mechanisms behind it are still not fully understood. In addition, stem cell therapy for IDD is still in the clinical trial stage, and there is still some distance from the final clinical application. For these reasons, we need to continue to conduct more in-depth research. Only by fully understanding these fields can we develop corresponding tissue engineering techniques to help MSCs better survive, proliferate, and synthesize ECM proteins in IVD tissues, so that stem cells can play a greater role in regeneration and repair in the treatment of IDD.

Data Availability

Data sharing is not applicable to this article as no new data was created or analyzed in this study.

Disclosure

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

All authors contributed to the conception of this work. Jing Zhang wrote the manuscript. Wentao Zhang revised the figure. Tianze Sun and Jinzuo Wang revised the tables. Ying Li, Jing Liu, and Zhonghai Li reviewed and revised the full text.

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