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**Barriers to mesenchymal stromal cells for low back pain**

MSCs for low back pain

## **Abstract**

Intervertebral disc degeneration is the main cause of low back pain. In the past 20 years, the injection of MSCs into the nucleus pulposus of the degenerative disc has become the main approach for the treatment of low back pain. Is this strategy feasible? Intervertebral disc is a highly complex load-bearing composite tissue composed of annulus fibrosus, nucleus pulposus and cartilaginous endplates. Any structural damage will change its overall biomechanical function, thereby causing progressive degeneration of the entire intervertebral disc. Therefore, treatment strategies should not only target the degenerated nucleus pulposus but also include degenerated annulus fibrosus or cartilaginous endplates. However, relatively few basic biological studies of the annulus fibrosus and cartilaginous endplates have been performed to date, although their pathological changes, such as annular tears or fissures, Modic changes, or Schmorl's nodes are more commonly associated with low back pain. Therefore, it remains an open question whether there are any regenerative techniques that can overcome the high load and harsh intervertebral disc microenvironment to achieve reparative regeneration of the annulus fibrosus or cartilage endplate. Furthermore, due to the harsh microenvironment of the degenerated intervertebral disc, the delivered MSCs die quickly. Taken together, current MSC-based regenerative medicine therapies to regenerate the entire disc complex by targeting the degenerated nucleus pulposus alone are unlikely to be successful. The success of disc repair depends on a comprehensive understanding of the basic cellular biology of the disc, as well as the pathophysiology and biomechanics following disc injury or degeneration, in order to develop more targeted regeneration techniques that anatomically and functionally replicate healthy native tissue.

**Key Words:** Intervertebral disc degeneration; Low back pain; Mesenchymal stromal cells

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**Core Tip:** Intervertebral disc is a highly complex weight-bearing tissue, and its degeneration is a major cause of low back pain. Current MSC-based clinical trials are difficult to succeed because the repair only targets the degenerated nucleus pulposus, and the transplanted cells die rapidly.

## **INTRODUCTION**

Low back pain is extremely common, affecting about 40% of the world's population, and is the most common cause of years lived with disability.<sup>1</sup> Its main cause is considered to be intervertebral disc degeneration (IDD), a chronic progressive process characterized by the loss of viable cells and the breakdown of the extracellular matrix within the disc, especially in its innermost region, the nucleus pulposus.<sup>2</sup> Therefore, the idea of transplanting living cells from patients or unrelated donors to supplement the nucleus pulposus cell population may be a possible solution to regenerate degenerate disc in vivo.<sup>3</sup> Many cell types, such as notochordal cells,<sup>4</sup> nucleus pulposus cells,<sup>5-7</sup> annulus fibrosus cells,<sup>8</sup> chondrocytes,<sup>9,10</sup> adult mesenchymal stromal cells (MSCs) from bone marrow,<sup>11,12</sup> adipose,<sup>13</sup> or umbilical cord,<sup>14</sup> embryonic stem cells,<sup>15,16</sup> or induced pluripotent stem cells,<sup>17-19</sup> have been used for regenerative therapy of degenerate discs. Among these cell types, MSCs, which exist in most stroma tissues, are heterogeneous populations containing pluripotent stem cells, progenitor cells and differentiated cells.<sup>20</sup> They provide an almost unlimited cell source with self-renewal ability and multilineage differentiation potential, and have become the most popular transplanted cells for intervertebral disc regeneration. Recently, various clinical trials have reported the application of MSCs, to repair and regenerate degenerate discs, whether alone or in combination with biomaterial scaffolds or carriers.<sup>2,3</sup> Despite the progress made in the field, much work remains to be done before MSC therapy become an effective new treatment.

### **IDD PATHOPHYSIOLOGY**

Both physiologically aged disc and pathologically painful disc show decreased signal intensity on magnetic resonance imaging T2-weighted images (black discs). The former is due to the increase in age, the activity of nucleus pulposus cells declines or the number decreases, resulting in the reduction of extracellular matrix, especially proteoglycan synthesis; the latter is the failure of the intervertebral disc structure, showing degenerative changes.<sup>21</sup> Pain provocation tests have demonstrated that painful intervertebral discs are always structurally ruptured.<sup>21,22</sup> Painful degenerative discs are characterized by the formation of vascularized granulation tissue that extends along a tear in the annulus fibrosus or a defective endplate into the nucleus pulposus, with extensive nociceptive innervation.<sup>22,23</sup> Once the disc ruptures, no matter in the annulus fibrosus or the endplate, the mechanical environment of the disc changes immediately, resulting in decompression of the nucleus pulposus and increased annulus fibrosus loading.<sup>21</sup> Different animal models of disc degeneration reveal that annular or endplate disruption inevitably leads to degenerative changes throughout the disc.<sup>24</sup> As the disc gradually degenerates, the nucleus pulposus shrinks and its hydrostatic pressure continues to drop, so more of the mechanical loading is borne by the annulus fibrosus.<sup>21</sup> In this case, attempts at cellular repair either in the annulus fibrosus or in the nucleus pulposus, become impossible, not because the cells are defective, but because their local mechanical environment is altered.

### **FEASIBILITY OF MSCS FOR IDD**

<sup>12</sup> The proteoglycan-rich gelatinous nucleus pulposus is sealed by the annulus fibrosus and cartilaginous endplates. The nucleus pulposus can perform its physiological function only when the annulus fibrosus and cartilaginous endplates are intact. Currently, treatment of IDD using MSCs refers to the delivery of MSCs to the nucleus pulposus region, thereby potentially repopulating the nucleus pulposus cells to repair the damaged disc or at least modulate the degenerative microenvironment.<sup>2,3</sup> Is this

treatment strategy feasible? First, we have to make it clear that the disc that needs MSC therapy is a painful disc, not an aging asymptomatic disc. Second, painful discs are always structurally defective, either in the annulus fibrosus or in the cartilaginous endplate. Discs with intact annulus fibrosus and cartilaginous endplates are always painless. Third, structural defects initiate degeneration of the entire disc including the nucleus pulposus, which is secondary. Even if the proteoglycan synthesis in the nucleus pulposus is increased by MSC injection, the mechanical environment of the nucleus pulposus is not restored because the defect of the annulus fibrosus or cartilaginous endplates is not repaired, and the partially regenerated nucleus pulposus will eventually degenerate further. Therefore, the treatment strategy should not only target the degenerated nucleus pulposus, but also include the defective annulus fibrosus or cartilaginous endplate.

<sup>8</sup> The torn annulus fibrosus heals defectively, possibly because of not only the inability of the sparse cell population to break down the large collagen fiber bundles of the annulus fibrosus and replace them with new ones, but also the poor blood supply.<sup>21</sup> The highly complex structure and composition of the annulus fibrosus endows it with excellent mechanical properties (Figure 1), making it challenging for tissue regeneration.<sup>25</sup> To date, little information is available on the basic cell biology of the annulus fibrosus, particularly regarding the origin of distinct cell populations and their functional roles, as well as phenotypic changes following injury or disease. Whether there are any regenerative techniques that can overcome the high load and harsh disc microenvironment to achieve reparative regeneration of the annulus fibrosus remains an open question.<sup>26</sup>

The cartilaginous endplate is a thin layer of hyaline cartilage that is structurally similar to articular cartilage. The research history of articular cartilage repair is much longer than that of intervertebral disc. Many attempts to regenerate cartilage have produced <sup>4</sup> hyaline-like tissue in vitro. In these techniques, a variety of cells <sup>4</sup> are capable of producing large amounts of proteoglycans and type II collagen. However, when tested *in vivo* in large animal models, none of these techniques restored the structure of the

collagen network, but instead formed fibrocartilage repair tissue, explaining their functional failure. The lack of progression in cartilage regeneration may be at least partly attributable to a focus on cell biology rather than biomechanics.<sup>27</sup> Given the importance of cartilaginous endplate in low back pain and nutrient supply to the disc, strategies aimed at restoring healthy cartilaginous endplate structure will prove critical. However, given the complexity of cartilaginous endplate repair and the limited understanding of its cell biology, current treatment strategies have not considered targeting cartilaginous endplates for low back pain.

### **MECHANISM OF ACTION OF MSCS FOR IDD**

It was originally envisioned that delivery of MSCs to the nucleus pulposus region within the degenerative disc will allow the cells to become nucleus pulposus cells under the influence of local signals and to replenish or regenerate the disc. Further research showed that the idea of MSCs as cell replacement therapy was almost entirely incorrect.<sup>28</sup> Several tracing studies involving intra-articular injection of labeled MSCs into rat and rabbit knee joints have shown that the number of cells delivered in the joints decreases rapidly after injection, and cells cannot be detected in the joints after several weeks. Similar to the ischemic microenvironment of infarcted heart, more than 99% of transplanted bone marrow MSCs cannot survive and die within 4 days after injection.<sup>29</sup> Thus, a large part of MSCs will die or apoptosis soon after transplantation to a degenerate disc.

Without an accurate understanding of the molecular mechanisms underlying the therapeutic effects of MSCs, it is impossible to consider the development of new medicinal products. According to the extensive literature over the past 20 years, 3 main mechanisms of action mediate the beneficial effects of MSCs after transplantation into recipients (Figure 2), including: (1) paracrine effects through soluble factors and extracellular vesicles (EVs); (2) mesodermal differentiation; (3) efferocytosis.<sup>28-30</sup> In most models tested, MSCs showed low levels of engraftment (usually 3% or less), accompanied by rapid clearance in large populations, but still had positive therapeutic



results, which seems to be related to the other two mechanisms.<sup>3</sup> The biological activity of MSCs in regeneration and immunomodulation is mediated by the paracrine release of biologically active factors and EVs, especially exosomes and microvesicles (MVs).<sup>1</sup> EVs are nanoscale vesicles actively released by cells and contain nucleic acids (DNA, mRNA and miRNA), proteins, metabolites and lipids; they are one of the most important means of cellular communication in vivo. EVs derived from MSCs<sup>1</sup> contribute to self-renewal, immunomodulation, expansion and damage repair of stem cells. Compared with MSC therapy, the application of MSC-EVs avoids the potential risk of stem cell transplantation and is more convenient for transportation and storage. At present, EVs derived from bone marrow mesenchymal stem cells have been proved to be a potential new therapy for a variety of diseases (including low back pain) in many experiments and clinical studies.<sup>31</sup> EVs can be classified into exosomes, MVs, and apoptotic bodies according to their biogenetics, size, and biophysical properties. MVs, also known as ectosomes, are heterogeneous membrane-bound vesicles with a diameter of 50-1000 nm<sup>9</sup> that play an important role in cell-cell communication, tissue homeostasis, cell differentiation, and organ development and remodeling.<sup>1</sup> Exosomes are small lipid membrane EVs formed by endocytosis, integration, and efflux; they are 30–150 nm in diameter.<sup>2</sup> Mediating cellular communication is the primary role of exosomes, as they can be released by one cell and captured by neighboring cells via ligand-receptor or direct binding.<sup>2</sup> MSC research has focused on MSC-derived exosomes (MSC-Exos). MSCs are the most prolific producers of exosomes, which share similar morphological characteristics, isolation methods, and preservation conditions as exosomes from other cell types. The composition of exosomes depends on their cellular origin. MSC-Exos have similar biological functions to MSCs, but are smaller in size, can penetrate biofilms, have low immunogenicity, and can be stored.<sup>32,33</sup> Functionally impaired, apoptotic, or dying MSCs may trigger phagocytosis by resident tissue macrophages, a process known as efferocytosis. This phenomenon is attributed to the polarization of macrophages towards<sup>3</sup> an anti-inflammatory phenotype and the release of soluble mediators such as interleukin-10, indoleamine 2,3-dioxygenase (IDO) and



transforming growth factor- $\beta$ , which ultimately leads to suppression or tolerance of immune effector cells.<sup>28</sup>

MSC therapies have been a hot topic in clinical trials for more than a generation, and the results of advanced clinical trials have failed to meet the expectations of encouraging preclinical animal data in various disease models.<sup>29</sup> In the field of MSC therapy, there is a contradiction between the effects of MSC manufactured by industrial MSCs and academic centers. Potential variables affecting MSCs based cell therapy include donor variance, *ex vivo* expansion and senescence, immunogenicity and cryopreservation.<sup>34</sup> Culture-expanded human MSCs showed potent immune T-, B-, and dendritic cell-targeted inhibitory properties through the expression of IDO and other effector molecules, many of which were enhanced by interferon- $\gamma$  stimulation. It is now well established that human MSCs licensed with interferon- $\gamma$  significantly enhance their immunosuppressive properties *in vitro* and that interferon- $\gamma$  responsiveness *in vivo* is essential for their suppressive function. Because interferon- $\gamma$  activates otherwise indistinguishable MSCS preparations from normal human donors, the magnitude of the IDO response varies considerably. Patients who receive MSCs from normal volunteers with low IFN- $\gamma$  response levels may have poorer results than patients who receive donor cells with high IFN- $\gamma$  response levels. A mechanistically defined, ideal MSCs immunoplastic profile could provide a scientific rationale for the selection of voluntary donors whose MSCs donation provides maximum veto function and avoids the pitfalls of injecting low-potency products into subjects participating in critical clinical trials.<sup>35</sup> In addition, culture-expanded human MSCs have been shown to experience telomere shortening and other phenotypic alterations that may play a role in modifying their regenerative and immunosuppressive properties. Among these acquired changes, MSCs gradually enter senescence as they approach replication failure. Although senescent MSCs have almost indistinguishable marker phenotypes from non-senescent MSCs, their functional properties, especially immune function, may be significantly altered.<sup>34,36,37</sup>

In many countries, a large number of unregulated clinics provide for-profit services with little or no oversight. These clinics often exaggerate the efficacy, but lack objective evidence.<sup>30</sup> Furthermore, industrial sponsors have led advanced phase III trials for nearly all MSC therapy, and the field as a whole has been heavily criticized for its ill-informed and irrational exuberance. Such criticism often reflects anxiety caused by the heavily predatory commercial activity of unregulated stem cell clinics around the world, leveraging the promise of unproven regenerative therapies, including MSCs, as a panacea.<sup>29</sup>

Most clinical trials of MSC therapy for low back pain published now show efficacy, including pain reduction and functional improvement, but insufficient evidence of improvement in disc structure.<sup>2</sup> Injected cells die rapidly due to immune-mediated damage and lack of nutrients and oxygen. The efficacy is likely to be related to the paracrine mechanism of MSCs. However, due to the short half-life of paracrine-produced soluble factors and mediators, the therapeutic effect of MSCs on IDD cannot be long-term or sustained. Intervertebral discs are prone to degeneration due to their avascular nature and low cell density. Human disc repair, including restoring disc height, is unlikely to be faster than in animals. The half-life of proteoglycans in human intervertebral disc is 3-6 years, whereas the half-life of fibrin such as collagen and elastin is over 50 years, reflecting the low rate of matrix synthesis and degradation in this tissue.<sup>38</sup> As mentioned earlier, the development of cell therapy regimens should target both the nucleus pulposus and the annulus fibrosus or cartilaginous endplates, but all cell-based clinical trials to date have focused on the nucleus pulposus, which is clearly flawed in terms of regenerative strategies. Furthermore, all cell treatments are unlikely to be successful until the biomechanical recovery of the degenerative disc.<sup>39</sup>

## **CONCLUSION**

The disc is a highly complex load-bearing mechanical device composed of three different cartilage structures, and any structural damage or degeneration of it alters its overall biomechanical function, so the repair strategy cannot only target the

degenerated nucleus pulposus. To date, relatively few basic biological studies have been performed on the annulus fibrosus and cartilaginous endplates, although their pathological changes such as annular tears or fissures, Modic changes, or Schmorl's nodes are more commonly associated with low back pain. Taken together, current MSC based regenerative medicine therapies to regenerate the entire disc complex by targeting the degenerated nucleus pulposus alone are unlikely to succeed. The success of disc repair depends on a comprehensive understanding of the basic cellular biology of the disc, and the pathophysiology and biomechanics following disc injury or degeneration, in order to develop more targeted regeneration techniques that anatomically and functionally replicate healthy native tissue.

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