Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: This study demonstrates the effect of mesenchymal stem cells on low back pain.
Response: Thank you very much for your hard work and time.

Reviewer #2:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: The manuscript discussed the possibility and potential mechanisms of MSCs therapy in intervertebral disc degeneration (IDD). Several issues should be addressed: 1. The authors should add a figure to illustrate the mechanisms of MSCs for alleviate IDD. 2. The authors have mentioned extracellular vesicles (EVs) and microvesicles (MVs), what's the different roles involving the MSCs biological activities.
Response: Thank you very much for your comments.

1. According to your suggestion, we have added a chart to illustrate the mechanism of MSC injection in treating low back pain.
2. In the revised manuscript, we have added the different biological roles of EVs and MVs originated from MSCs. EVs derived from MSCs 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. MVs, also known as ectosomes, are heterogeneous membrane-bound vesicles with a diameter of 50-1000 nm that play an important role in cell-cell communication, tissue homeostasis, cell differentiation, and organ development and remodeling. (Shen Q, Huang Z, Yao J, Jin Y. Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. J Adv Res. 2021 Jul 8;37:221-233.)

Reviewer #3:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision
Specific Comments to Authors: The authors have to provide a detailed and proper definition for human mesenchymal stem cells (MSCs). They define these cells as stem cells, but the proper definition is stromal cells. The authors should also better explain that the isolation of MSCs, according to current criteria, produces heterogeneous,

non-clonal cultures of stromal cells containing stem cells with different multipotential properties, committed progenitors and differentiated cells (PMID: 34398443). There are several issues related to MSC transplants that authors should address. For example, in recent years, some investigators carried out meta-analysis studies to identify the potential variables affecting cellular therapies based on MSCs. Donor variance, ex vivo expansion and senescence, immunogenicity and cryopreservation are among the main factors that can compromise the effectiveness of MSC transplants. Immunoregulatory properties of MSCs may have a significant inter-donor variability. Interferon-gamma-induced IDO (Indoleamine 2,3-Dioxygenase) upregulation may be used as a marker of immunosuppression activity. The authors should consider this issue. See for for example, Galipeau addressed the importance of senescence in failure of stem cell-based trials (Cytotherapy. 2013 Jan;15(1):2-8. doi: 10.1016/j.jcyt.2012.10.002. The mesenchymal stromal cells dilemma--does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road?)
Response: Thank you very much for your detailed comments.

According to your comments, we have changed mesenchymal stem cells into mesenchymal stromal cells in the revised manuscript, and added the definition of mesenchymal stromal cells. Among these cell types, MSCs, which exist in most stroma tissues, are heterogeneous populations containing pluripotent stem cells, progenitor cells and differentiated cells.(Galderisi U, Peluso G, Di Bernardo G. Clinical Trials Based on Mesenchymal Stromal Cells are Exponentially Increasing: Where are We in Recent Years? Stem Cell Rev Rep. 2022;18(1):23-36.) 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.

According to your suggestion, we have added the following contents to the revised manuscript:

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. (Galipeau J. The mesenchymal stromal cells dilemma--does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? Cytotherapy. 2013;15(1):2-8.)

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-γ stimulation. It is now well established that human MSCs licensed with interferon-γ significantly enhance their immunosuppressive properties in vitro and that interferon-γ responsiveness in vivo is

essential for their suppressive function. Because interferon-γ 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-γ response levels may have poorer results than patients who receive donor cells with high IFN-γ 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. (Francois M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther. 2012;20:187e95.)

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. (Digirolamo CM, Stokes D, Colter D, Phinney DG, Class R, Prockop DJ. Propagation and senescence of human marrow stromal cells in culture: a simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate. Br J Haematol. 1999;107:275e81.
Bork S, Pfister S, Witt H, Horn P, Korn B, Ho AD, et al. DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. Aging Cell. 2010;9:54e63.)