# Cell-free Therapy for Inflammatory Diseases: Opportunities and Challenges

**Article** *in* Recent Patents on Inflammation & Allergy Drug Discovery · December 2021

DOI: 10.2174/2772270816666211220152218



**5 authors**, including:

Sharun Khan
Indian Veterinary Research Institute
**225** PUBLICATIONS **3,814** CITATIONS
SEE PROFILE

Kuldeep Dhama
Indian Veterinary Research Institute
**1,122** PUBLICATIONS **23,268** CITATIONS
SEE PROFILE

Kaveri M Jambagi
Indian Veterinary Research Institute
**17** PUBLICATIONS **11** CITATIONS
SEE PROFILE

Amarpal Amarpal
Indian Council of Agricultural Research
**404** PUBLICATIONS **2,398** CITATIONS
SEE PROFILE

Sharun K, Dhama K, Jambagi K, Pawde AM, Amarpal. Cell-free therapy for inflammatory diseases: Opportunities and challenges. Recent Adv Inflamm Allergy Drug Discov. 2021 Dec 20. doi: 10.2174/2772270816666211220152218. Epub ahead of print. PMID: 34931977.1

Editorial

# Cell-free therapy for inflammatory diseases: Opportunities and challenges

Khan Sharun[1*], Kuldeep Dhama[2*], Kaveri Jambagi[3], Abhijit M. Pawde[1], Amarpal[1]

[1]Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

[2]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

[3]Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India.

***Corresponding Authors:**

Khan Sharun, MVSc.
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India. Email: sharunkhansk@gmail.com / sharunkhan@ivri.res.in

Kuldeep Dhama, Ph.D.
Division of Pathology, ICAR –Indian Veterinary Research Institute, Izatnagar, Bareilly, India. Email: kdhama@rediffmail.com

**Word count**: 1211 | **Pages**: 7 | **References**: 27 | **Figures**: 2

**Running title**: Cell-free therapy for inflammatory diseases

**Keywords:** stem cell therapy; mesenchymal stem cells; cell-free therapy; inflammatory diseases; regenerative medicine; extracellular vesicle

Sharun K, Dhama K, Jambagi K, Pawde AM, Amarpal. Cell-free therapy for inflammatory diseases: Opportunities and challenges. Recent Adv Inflamm Allergy Drug Discov. 2021 Dec 20. doi: 10.2174/2772270816666211220152218. Epub ahead of print. PMID: 34931977.2

Mesenchymal stem cells (MSCs) are widely used as a therapeutic agent for managing acute and chronic inflammatory diseases in humans and animals [1,2,3,4]. The immunomodulatory effects of MSCs help regulate inflammation, thereby promoting the repair of damaged tissues [3,4,5]. MSCs are isolated from various fetal and adult tissues like adipose tissue, bone marrow, peripheral blood, umbilical cord blood, placenta, dental pulp, endometrium, and skeletal muscle [6]. It was initially hypothesized that MSCs produce an effect in the injured tissue by differentiating into specialized cells. However, later studies confirmed that only a small proportion of the transplanted cells survive and differentiate in host tissues [5]. The majority of the therapeutic potential exhibited by MSCs is due to the paracrine effect on host cells mediated using cytokines, exosomes, growth factors, and extracellular matrix molecules [4,7]. These mediators promote the repair and regeneration of injured tissues by modulating cell differentiation, angiogenesis, and endogenous cell migration [4]. The therapeutic potential of MSCs (angiogenic, anti-inflammatory, anti-oxidant, and anti-fibrotic activity) are produced by cytokines, chemokines, trophic factors, and EVs [7,8]. However, cell-based therapy is associated with several shortcomings, including a limit on the number of cells that can be administered and their inability to reach target organs [8].

Extracellular vesicle (EV) is the common term used to define all lipid-bilayer bound cell-derived vesicles (Figure 1). The term was first defined by the International Society for Extracellular Vesicles (ISEV). Other terms used to describe EVs are exosomes, prostasomes, ectosomes, microvesicles, and matrix vesicles [9]. According to vesicle morphology (mainly size), EVs are classified into apoptotic bodies (diameters ranging from 800-5000 nm), microvesicles (50-1000 nm), and exosomes (30-150 nm) [10]. The contents of MSC-derived exosomes vary and include growth factors, cytokines, regulatory miRNAs, mRNAs, and signaling lipids [11,12,13]. The content varies depending on the tissue of origin, immediate intercellular neighbors of the MSCs, and can be altered by culturing in a specific microenvironment [11,12]. They also transport genetic materials from MSCs to host cells and are involved in intercellular communication [14]. Exosomes can be considered an attractive alternative to cell therapy due to the flexibility offered by these biological vesicles that allow modifying their cargo [15]. Depending on the therapeutic application, they can be modified to carry specific drugs, RNA, and proteins [10,15]. In addition, the surface receptors of the exosome membrane can be engineered to target specific lesions [15].

Cell-free therapy has great prospects in osteoarthritis management. The paracrine effects of MSC-derived exosomes can promote cartilage regeneration. In addition, the exosomes can also be used as a delivery vehicle for osteoarthritis treatment [16]. Exosome-based therapeutic strategies are being developed for managing acute respiratory distress syndrome (ARDS) associated with Coronavirus disease (COVID-19). This includes using MSCs-secretome, exosomes incorporating specific mRNAs and miRNAs, and exosomes as carriers of drugs [17,18]. Some of these strategies are being evaluated in clinical trials worldwide. MSC-derived extracellular vesicles (MSC-EV) have therapeutic benefits in sepsis and could be considered an alternative to cell-based therapies [19]. MSC-derived exosomes

also possess the ability to home preferentially to inflamed tissues and suppress inflammatory responses [11,14,20].

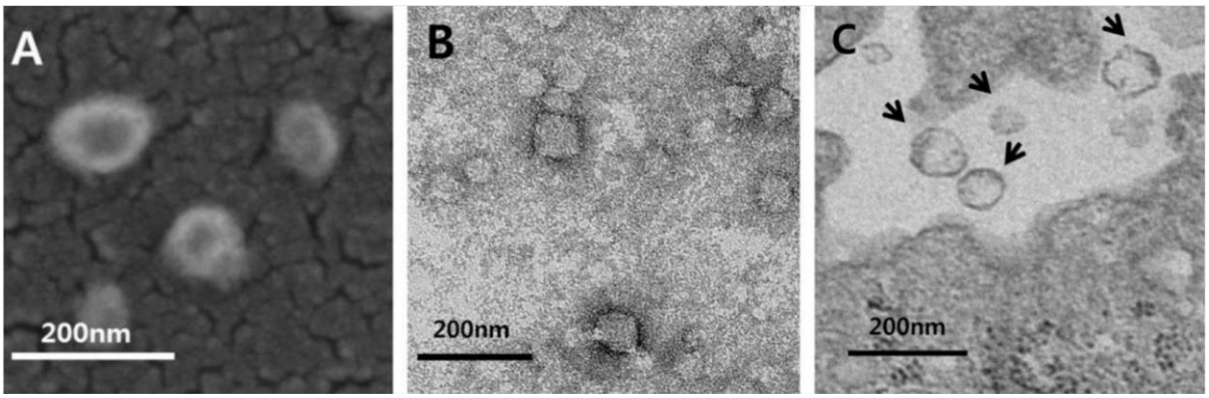


**Figure 1**: Mesenchymal stem cell (MSC) derived extracellular vesicles (EVs). **(A)** Scanning electron microscopic image of EVs isolated from the conditioned media of human umbilical cord blood-derived MSC using ultra-centrifugation. **(B)** Transmission electron microscope (TEM) image of EVs. **(C)** TEM image of EVs being secreted from an MSC membrane. Reproduced from Sung et al. [21] under Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Burn injury induces an inflammatory reaction associated with increased TNF-α and IL-1β levels and decreased IL-10 levels. This can be successfully reversed by administering human umbilical cord MSC-derived exosomes in rats. The higher expression of miR-181c in the exosomes played a pivotal role in reducing burn-induced inflammation by downregulating the Toll-like receptor 4 (TLR4) signaling pathway [22]. Similarly, umbilical cord MSC-derived exosomes-mimetic nanovesicles were successfully used to attenuate TNF-α-induced inflammation in endothelial cells. In addition, the expression of pro-inflammatory cytokines like TNF-α, IL-1β, IL-6, and IL-8 was reduced [23]. Furthermore, MSC-derived exosome miR-542-3p was found to prevent ischemia-induced glial cell inflammatory response by inhibiting the TLR4 signaling pathway. Therefore, the delivery of miR-542-3p using exosomes might help to treat cerebral ischemic injury [24]. Adipose-derived MSC-secreted exosomes were successfully used to alleviate dextran sulfate sodium-induced acute colitis in mice. MSC-derived exosome can regulate the Regulatory T cells (Tregs) population and decrease inflammatory cytokine production [25]. In addition, adipose-derived MSC-secreted exosomes possess immunomodulatory, proangiogenic, neurotrophic, and epithelization activity that can be used for managing respiratory, cardiovascular, neurodegenerative, inflammatory, and autoimmune diseases [26]. Allergic rhinitis is a type I allergic disease affecting nasal mucosa due to allergen exposure. Bone marrow MSC-derived secretome is a promising treatment for managing allergic rhinitis via the paracrine mechanism [27].

MSC-EVs have several advantages such as high stability, being suitable for long-term storage, superior safety profile, lacks self-replicating capabilities, and ectopic differentiation, lacks tumorigenicity, poorly immunogenic (even in allogeneic application), and absence of pulmonary embolism (Figure 2) [7,19]. Due to the smaller diameter of EVs, the risk of

thrombosis associated with intravenous administration is negligible compared to that posed by stem cells [8]. Although several studies are conducted to evaluate the therapeutic potential of exosomes, no unified standard is available for the standardization and characterization of different exosomes for clinical use [15]. In addition, there is no consensus regarding the markers that can be used to distinguish different categories of EVs secreted from the cells [8,19]. Cell-free therapy is still in its infancy, and therefore relevant medico-legal norms concerning their clinical use are not yet sufficiently established [8]. Further studies are required to optimize the methods for producing MSC-EVs on a large scale, evaluate their half-life and dosage for clinical applications [10,19].

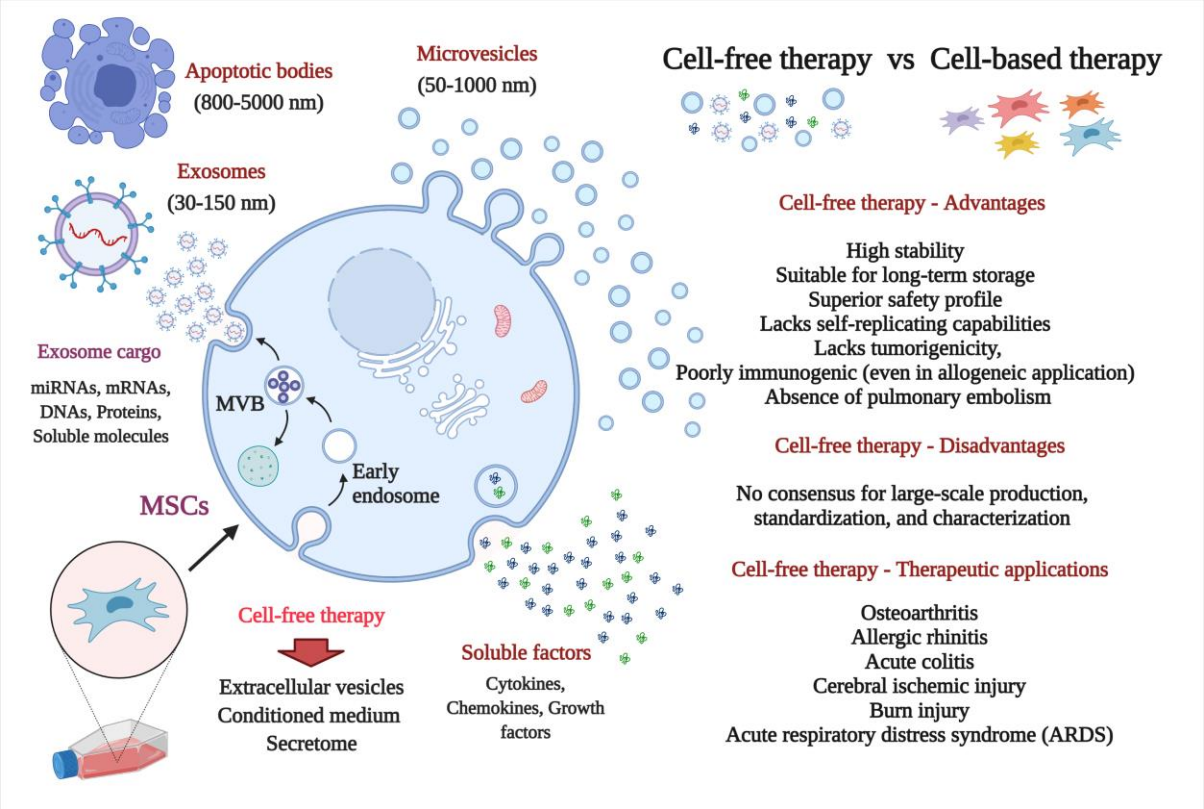


**Figure 2**: Illustrated the different mesenchymal stem cell (MSC) derived extracellular vesicles and the characteristics of cell-free therapy.

Cell-free therapy is the future of MSCs based therapeutics as it overcomes the major limitations of cell-based therapy, including tumor differentiation and embolization. The future of MSC-based therapeutics is expected to be dominated by MSC-EVs. They are the key messenger between MSCs and damaged tissues and operate similar to MSCs but lack the disadvantages of cell-based therapies. MSC-EVs have broad therapeutic applications in managing inflammatory diseases due to their potential for modulating the immune system and suppress inflammatory responses. However, several challenges are to be addressed concerning the standardization and characterization of different MSC-EVs products. This will ensure the successful translation of MSC-EV-based cell-free therapy in clinical practice.

Sharun K, Dhama K, Jambagi K, Pawde AM, Amarpal. Cell-free therapy for inflammatory diseases: Opportunities and challenges. Recent Adv Inflamm Allergy Drug Discov. 2021 Dec 20. doi: 10.2174/2772270816666211220152218. Epub ahead of print. PMID: 34931977.5

**Acknowledgments:** None

**Funding:** The authors received no funding in relation to this article.

**Declaration of Interest:** All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

**Author contributions:** KS conceptualized the manuscript; KS wrote the first draft with input from KD, KJ, AMP, and Amarpal; all authors contributed to revisions and approved the final manuscript.

## References

1. Amarpal, Dhama K, Chakraborty S, Tiwari R, Natesan S. Stem cells and their clinical/therapeutic applications in biomedical and veterinary science – The perspectives. Res. Opin. Anim. Vet. Sci. 2013; 3(9): 261-279.
2. Gugjoo MB, Amarpal, Chandra V, Wani MY, Dhama K, Sharma GT. Mesenchymal Stem Cell Research in Veterinary Medicine. Curr Stem Cell Res Ther. 2018;13(8):645-657. doi: 10.2174/1574888X13666180517074444. PMID: 29769009
3. Gugjoo MB, Hussain S, Amarpal, Shah RA, Dhama K. Mesenchymal Stem Cell-Mediated Immuno-Modulatory and Anti- Inflammatory Mechanisms in Immune and Allergic Disorders. Recent Pat Inflamm Allergy Drug Discov. 2020;14(1):3-14. doi: 10.2174/1872213X14666200130100236. PMID: 32000656; PMCID: PMC7509741
4. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nat Rev Nephrol. 2018 Aug;14(8):493-507. doi: 10.1038/s41581-018-0023-5. PMID: 29895977.
5. Baglio SR, Pegtel DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. Front Physiol. 2012 Sep 6;3:359. doi: 10.3389/fphys.2012.00359. PMID: 22973239; PMCID: PMC3434369.
6. Berebichez-Fridman R, Montero-Olvera PR. Sources and Clinical Applications of Mesenchymal Stem Cells: State-of-the-art review. Sultan Qaboos Univ Med J. 2018 Aug;18(3):e264-e277. doi: 10.18295/squmj.2018.18.03.002. Epub 2018 Dec 19. PMID: 30607265; PMCID: PMC6307657.
7. Jafarinia M, Alsahebfosoul F, Salehi H, Eskandari N, Ganjalikhani-Hakemi M. Mesenchymal Stem Cell-Derived Extracellular Vesicles: A Novel Cell-Free Therapy. Immunol Invest. 2020 Oct;49(7):758-780. doi: 10.1080/08820139.2020.1712416. Epub 2020 Feb 2. PMID: 32009478.
8. Watanabe Y, Tsuchiya A, Terai S. The development of mesenchymal stem cell therapy in the present, and the perspective of cell-free therapy in the future. Clin Mol Hepatol. 2021 Jan;27(1):70-80. doi: 10.3350/cmh.2020.0194. Epub 2020 Dec 3. PMID: 33317249; PMCID: PMC7820202.
9. Malda J, Boere J, van de Lest CH, van Weeren P, Wauben MH. Extracellular vesicles - new tool for joint repair and regeneration. Nat Rev Rheumatol. 2016

Apr;12(4):243-9. doi: 10.1038/nrrheum.2015.170. PMID: 26729461; PMCID: PMC7116208.

10. Phan J, Kumar P, Hao D, Gao K, Farmer D, Wang A. Engineering mesenchymal stem cells to improve their exosome efficacy and yield for cell-free therapy. J Extracell Vesicles. 2018 Sep 26;7(1):1522236. doi: 10.1080/20013078.2018.1522236. PMID: 30275938; PMCID: PMC6161586.
11. Pashoutan Sarvar D, Shamsasenjan K, Akbarzadehlaleh P. Mesenchymal Stem Cell-Derived Exosomes: New Opportunity in Cell-Free Therapy. Adv Pharm Bull. 2016 Sep;6(3):293-299. doi: 10.15171/apb.2016.041. Epub 2016 Sep 25. PMID: 27766213; PMCID: PMC5071792.
12. Phinney DG, Pittenger MF. Concise Review: MSC-Derived Exosomes for Cell-Free Therapy. Stem Cells. 2017 Apr;35(4):851-858. doi: 10.1002/stem.2575. Epub 2017 Mar 10. Erratum in: Stem Cells. 2017 Sep;35(9):2103. PMID: 28294454.
13. Peng D, Yang X, Zhang L, Song X, Jiang T, Hui Y, et al. Cell-Free Therapy may Experience More Rapid Advancement "Pretended Bystander Effects" in Cell-Based Therapy for Treating Diseases. Turk Neurosurg. 2020;30(2):315-316. doi: 10.5137/1019-5149.JTN.27196-19.2. PMID: 32091116.
14. Cheng L, Zhang K, Wu S, Cui M, Xu T. Focus on Mesenchymal Stem Cell-Derived Exosomes: Opportunities and Challenges in Cell-Free Therapy. Stem Cells Int. 2017;2017:6305295. doi: 10.1155/2017/6305295. Epub 2017 Dec 19. PMID: 29410682; PMCID: PMC5749272.
15. Skuratovskaia D, Vulf M, Khaziakhmatova O, Malashchenko V, Komar A, Shunkin E, et al. Exosome Limitations in the Treatment of Inflammatory Diseases. Curr Pharm Des. 2020 Dec 10. doi: 10.2174/1381612826666201210120444. Epub ahead of print. PMID: 33302851.
16. Duan L, Xu X, Xu L, Chen H, Li X, Alahdal M, et al. Exosome-mediated drug delivery for cell-free therapy of osteoarthritis. Curr Med Chem. 2020 Nov 18. doi: 10.2174/0929867327666201118161232. Epub ahead of print. PMID: 33213308.
17. Rezakhani L, Kelishadrokhi AF, Soleimanizadeh A, Rahmati S. Mesenchymal stem cell (MSC)-derived exosomes as a cell-free therapy for patients Infected with COVID-19: Real opportunities and range of promises. Chem Phys Lipids. 2021 Jan;234:105009. doi: 10.1016/j.chemphyslip.2020.105009. Epub 2020 Nov 12. PMID: 33189639; PMCID: PMC7658620.
18. Singh B, Mal G, Verma V, Tiwari R, Khan MI, Mohapatra RK, et al. Stem cell therapies and benefaction of somatic cell nuclear transfer cloning in COVID-19 era. Stem Cell Res Ther. 2021 May 12;12(1):283. doi: 10.1186/s13287-021-02334-5. PMID: 33980321; PMCID: PMC8114669.
19. Cheng Y, Cao X, Qin L. Mesenchymal Stem Cell-Derived Extracellular Vesicles: A Novel Cell-Free Therapy for Sepsis. Front Immunol. 2020 Apr 21;11:647. doi: 10.3389/fimmu.2020.00647. PMID: 32373121; PMCID: PMC7186296.
20. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci. 2014 Mar 7;15(3):4142-57. doi: 10.3390/ijms15034142. PMID: 24608926; PMCID: PMC3975389.
21. Sung DK, Chang YS, Sung SI, Ahn SY, Park WS. Thrombin Preconditioning of Extracellular Vesicles Derived from Mesenchymal Stem Cells Accelerates Cutaneous Wound Healing by Boosting Their Biogenesis and Enriching Cargo

Content. J Clin Med. 2019 Apr 18;8(4):533. doi: 10.3390/jcm8040533. PMID: 31003433; PMCID: PMC6517934.

22. Li X, Liu L, Yang J, Yu Y, Chai J, Wang L, et al. Exosome Derived From Human Umbilical Cord Mesenchymal Stem Cell Mediates MiR-181c Attenuating Burn-induced Excessive Inflammation. EBioMedicine. 2016 Jun;8:72-82. doi: 10.1016/j.ebiom.2016.04.030. Epub 2016 Apr 27. PMID: 27428420; PMCID: PMC4919539.
23. Ko KW, Yoo YI, Kim JY, Choi B, Park SB, Park W, et al. Attenuation of Tumor Necrosis Factor-α Induced Inflammation by Umbilical Cord-Mesenchymal Stem Cell Derived Exosome-Mimetic Nanovesicles in Endothelial Cells. Tissue Eng Regen Med. 2020 Apr;17(2):155-163. doi: 10.1007/s13770-019-00234-7. Epub 2020 Feb 5. PMID: 32026314; PMCID: PMC7105540.
24. Cai G, Cai G, Zhou H, Zhuang Z, Liu K, Pei S, et al. Mesenchymal stem cell-derived exosome miR-542-3p suppresses inflammation and prevents cerebral infarction. Stem Cell Res Ther. 2021 Jan 6;12(1):2. doi: 10.1186/s13287-020-02030-w. PMID: 33407827; PMCID: PMC7786953.
25. Heidari N, Abbasi-Kenarsari H, Namaki S, Baghaei K, Zali MR, Ghaffari Khaligh S, et al. Adipose-derived mesenchymal stem cell-secreted exosome alleviates dextran sulfate sodium-induced acute colitis by Treg cell induction and inflammatory cytokine reduction. J Cell Physiol. 2021 Aug;236(8):5906-5920. doi: 10.1002/jcp.30275. Epub 2021 Mar 16. PMID: 33728664.
26. Trzyna A, Banaś-Ząbczyk A. Adipose-Derived Stem Cells Secretome and Its Potential Application in "Stem Cell-Free Therapy". Biomolecules. 2021 Jun 13;11(6):878. doi: 10.3390/biom11060878. PMID: 34199330; PMCID: PMC8231996.
27. Zou W, Liu G, Zhang J. Secretome from bone marrow mesenchymal stem cells: A promising, cell-free therapy for allergic rhinitis. Med Hypotheses. 2018 Dec;121:124-126. doi: 10.1016/j.mehy.2018.09.016. Epub 2018 Sep 10. PMID: 30396464.