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Stem Cells and Cellular Therapy International

Official Journal of Indian Stem Cell Study Group Association

Journal homepage: https://sccti.in/

Review Article

Mesenchymal Stem Cells (MSCs) conditioned medium for regenerative medicine: Mechanisms, applications, and translational perspectives

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Abstract

Mesenchymal stem cells (MSCs) have been extensively investigated for their regenerative and immunomodulatory properties. While early therapeutic strategies focused on the engraftment and differentiation of transplanted MSCs, growing evidence indicates that their clinical benefits are predominantly mediated through paracrine mechanisms. These paracrine factors, collectively referred to as the MSC secretome, are present in mesenchymal stem cells–conditioned medium (MSC-CM) and include cytokines, growth factors, chemokines, and extracellular vesicles. MSC-CM has emerged as a promising cell-free therapeutic alternative that retains the regenerative, angiogenic, anti-apoptotic, and immunomodulatory functions of MSCs while overcoming many safety and logistical challenges associated with cell-based therapies. This review comprehensively summarizes the composition, mechanisms of action, and regenerative applications of MSC-CM across various organ systems, including musculoskeletal, cutaneous, neurological, and ocular tissues. Particular emphasis is placed on the role of Wharton's jelly–derived MSCs, long-term in vitro expansion, and immunomodulatory properties relevant to inflammatory and cytokine storm–associated conditions. Current challenges and future directions for clinical translation of MSC-CM are also discussed.

Keywords: Mesenchymal stem cells, Conditioned medium, MSC secretome, Regenerative medicine, Immunomodulation, Wharton's jelly-derived MSCs, Cell-free therapy

Received: 05-01-2026; **Accepted:** 28-02-2026; **Available Online:** 09-03-2026

1. Introduction

Mesenchymal stem cells (MSCs) are adult multipotent stromal cells capable of differentiating into mesodermal lineages such as osteoblasts, chondrocytes, and adipocytes. Initially, their therapeutic application in regenerative medicine was attributed to their engraftment and differentiation at injury sites. However, accumulating evidence over the past two decades has demonstrated that MSC-mediated tissue repair occurs predominantly through paracrine mechanisms rather than direct cell replacement.[1,2,3]

This paradigm shift has led to increasing interest in the MSC secretome, which includes soluble proteins, cytokines, chemokines, growth factors, lipids, nucleic acids, and extracellular vesicles (EVs), including exosomes. The culture supernatant enriched with these bioactive molecules is referred to as MSC-conditioned medium (MSC-CM). MSC-CM represents a cell-free therapeutic alternative that retains most regenerative and immunomodulatory properties of MSCs while overcoming many limitations associated with live cell transplantation.[4,5]

1.1. Composition of MSC-conditioned medium

MSC-CM contains a complex and dynamic mixture of bioactive factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), stromal cell-derived factor-1 (SDF-1), prostaglandin E2 (PGE2), and tumor necrosis factor-stimulated gene-6 (TSG-6).[6,7,8] **(Table 1)**

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Table 1: Major bioactive components of mesenchymal stem cells conditioned medium and their biological functions

Component	Source	Biological function
Vascular Endothelial Growth Factor (VEGF)	MSCs	Angiogenesis, endothelial cell proliferation
Hepatocyte Growth Factor (HGF)	MSCs	Anti-apoptotic, tissue regeneration
Insulin-like Growth Factor-1 (IGF-1)	MSCs	Cell survival, proliferation
Fibroblast Growth Factor (FGF)	MSCs	Cell migration, angiogenesis
Platelet-Derived Growth Factor (PDGF)	MSCs	Tissue repair, ECM remodeling
Transforming Growth Factor-β (TGF-β)	MSCs	Immunomodulation, fibrosis regulation
Interleukin-10 (IL-10)	MSCs	Anti-inflammatory effects
Prostaglandin E2 (PGE2)	MSCs	Immune suppression, macrophage polarization
Tumor Necrosis Factor-stimulated Gene-6 (TSG-6)	MSCs	Anti-inflammatory, tissue protection
Extracellular vesicles (exosomes)	MSCs	Gene regulation, intercellular communication

Table 2: Comparison of mesenchymal stem cell sources for conditioned medium production

MSC Source	Advantages	Limitations
Bone marrow-derived MSCs	Well characterized, extensive clinical data	Invasive collection, donor variability
Adipose tissue-derived MSCs	High cell yield, minimally invasive	Variable secretome composition
Wharton's jelly-derived MSCs	High proliferation, immunoprivileged, stable phenotype, non-invasive procurement	Limited long-term clinical trials

In addition, MSC-CM is rich in extracellular vesicles that carry functional proteins, mRNA, microRNAs, and long non-coding RNAs capable of modulating gene expression in recipient cells. Proteomic and transcriptomic analyses have revealed that the composition of MSC-CM varies depending on the tissue source of MSCs, donor characteristics, passage number, and culture conditions. This complexity enables MSC-CM to exert pleiotropic effects on tissue repair and immune regulation.

In vitro expansion of MSCs is necessary for generating clinically relevant quantities of conditioned medium. However, prolonged passaging is associated with replicative senescence, genomic instability, altered differentiation potential, and functional decline due to in vitro aging.

Although MSC immunophenotypic markers (CD73, CD90, CD105 positivity; hematopoietic marker negativity) may persist with passaging, surface marker stability does not guarantee functional potency, safety, or therapeutic efficacy. Furthermore, pluripotency-associated gene expression typically decreases with successive passages.

Regulatory frameworks, including recommendations aligned with ICMR guidelines, generally restrict clinical use of MSCs to early passages (preferably ≤ P6). Several studies evaluating Wharton's jelly–derived MSCs (WJ-MSCs) suggest limiting expansion to fewer than 8–10 passages to minimize senescence-associated alterations. Therefore, MSC-CM intended for translational application should be generated from limited-passage, well-characterized parental MSCs, with defined potency assays and senescence monitoring.

Beyond soluble factors, the quality, stability, and biological potency of MSC-conditioned medium are directly dependent on the phenotypic integrity and expansion characteristics of the parental MSCs. In this context, Wharton's Jelly–derived MSCs (WJ-MSCs) have gained considerable attention due to their primitive phenotype, high proliferative capacity, and immunological immaturity. **(Table 2), (Figure 1)**

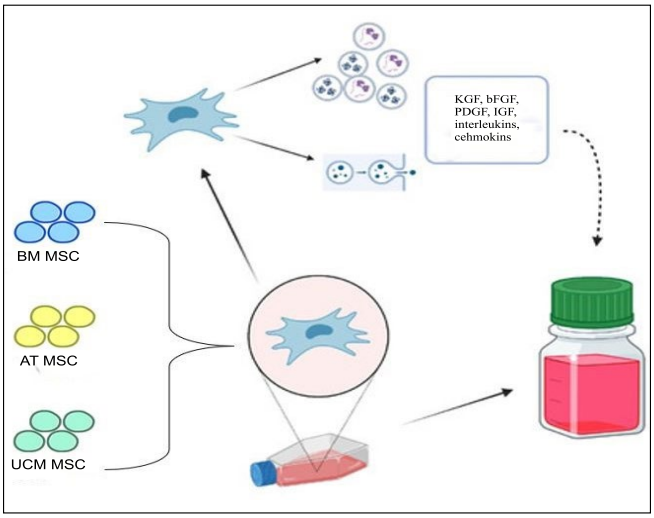

Figure 1: Production mesenchymal stem cell conditioned medium, which contains secreted factors from source stem cells

Panwar et al. demonstrated that in vitro expanded WJ-MSCs retain stable morphology, surface marker expression, genetic integrity, and differentiation potential, supporting their suitability for clinical-grade applications.[9] This is particularly relevant for MSC-CM–based therapies, as MSCs culture is often required to generate sufficient quantities of conditioned medium without compromising safety or efficacy

Further molecular characterization by Panwar and Mishra et al. confirmed the robust expression of MSC-specific surface markers (CD73, CD90, CD105) and Pluripotency associated

genes in WJ-MSCs, while lacking hematopoietic markers, validating their identity and functional competence.[10] Such rigorous characterization strengthens the translational reliability of MSC-derived secretome products.

1.3. Paracrine mechanisms and regenerative actions

The regenerative potential of MSC-CM is largely mediated through angiogenesis, anti-apoptotic signaling, cell proliferation, migration, and extracellular matrix remodeling. VEGF, angiopoietin-1, and PDGF present in MSC-CM promote endothelial cell proliferation and neovascularization, which are critical for tissue regeneration.[11]

MSC-CM has also been shown to inhibit apoptosis by activating survival pathways such as PI3K/Akt and ERK1/2 while suppressing oxidative stress and mitochondrial dysfunction in injured tissues. Furthermore, MSC-CM enhances the recruitment and activation of endogenous progenitor cells, thereby amplifying intrinsic repair mechanisms without the need for cell engraftment.

1.4. Immunomodulatory and anti-inflammatory effects

One of the most extensively studied properties of MSC-CM is its immunomodulatory capacity. MSC-CM suppresses pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 while promoting anti-inflammatory mediators like IL-10 and TGF-β.[12]

MSC-CM modulates both innate and adaptive immune responses by inhibiting dendritic cell maturation, suppressing T-cell proliferation, inducing regulatory T cells, and promoting macrophage polarization toward the anti-inflammatory M2 phenotype.[13] These properties make MSC-CM particularly attractive for treating inflammatory, autoimmune, and ischemic conditions where excessive immune activation hampers regeneration.

These immunomodulatory effects have gained particular relevance in hyperinflammatory conditions. The role of MSCs and their secretome in mitigating cytokine storm syndromes, such as those observed during COVID-19 infection, highlights the potential of MSC-CM as a safe and effective cell-free immunotherapeutic strategy.

The immunomodulatory capacity of MSC-CM has gained heightened clinical relevance in hyperinflammatory conditions, including cytokine storm syndromes. A comprehensive review by Panwar and Mishra highlighted the potent immunosuppressive and immune balancing effects of MSCs, emphasizing their ability to modulate excessive cytokine release through paracrine signaling.[14]

MSC-derived secretome components such as IL-10, TGF-β, PGE2, and indoleamine 2,3-dioxygenase (IDO) play a pivotal role in attenuating pro-inflammatory cascades, inhibiting Th1 and Th17 responses, and promoting regulatory T-cell expansion. These mechanisms were particularly underscored during the COVID-19 pandemic, where MSC-based and MSC-CM–based approaches emerged as promising adjunct therapies for managing cytokine storm–associated tissue damage.[14]

These findings reinforce the rationale for MSC-CM as a safer, cell-free immunomodulatory therapeutic strategy, especially in systemic inflammatory and immune-mediated disorders.

1.5. Role of MSC-CM in bone and musculoskeletal regeneration

Numerous preclinical studies have demonstrated that MSC-CM enhances osteogenesis and bone repair by stimulating osteoblast proliferation, differentiation, and mineralization. Growth factors such as BMP-2, IGF-1, and VEGF present in MSC-CM contribute to bone remodeling and vascularization.

Systematic reviews and meta-analyses have confirmed that MSC-CM significantly improves bone healing outcomes in animal models, including critical-sized defects. Beyond bone, MSC-CM has shown efficacy in cartilage repair, tendon healing, and skeletal muscle regeneration, highlighting its broad musculoskeletal applications.

1.6. MSC-CM in wound healing and skin regeneration

MSC-CM has been widely investigated in cutaneous wound healing models. It accelerates wound closure by enhancing keratinocyte and fibroblast proliferation, promoting angiogenesis, and improving collagen organization.

Additionally, MSC-CM reduces scar formation by modulating fibroblast activity and inhibiting excessive myofibroblast differentiation. Studies comparing MSC-CM derived from bone marrow, adipose tissue, and umbilical cord indicate that umbilical cord-derived MSC-CM exhibits superior angiogenic and epithelial regenerative effects.

Comparative studies suggest that conditioned medium derived from WJ-MSCs exhibits superior epithelial regeneration and angiogenic potential compared to adult tissue-derived MSCs, making it particularly attractive for dermatological and reconstructive applications.

1.7. Neurological and ocular applications

In neurological disorders, MSC-CM has demonstrated neuroprotective and neuroregenerative effects by reducing neuroinflammation, promoting neurite outgrowth, and enhancing synaptic plasticity. Animal models of spinal cord injury, stroke, and neurodegenerative diseases have shown improved functional recovery following MSC-CM administration.

Similarly, in ophthalmology, MSC-CM has shown promise in treating corneal injuries, retinal degeneration, and optic nerve damage by inhibiting apoptosis, reducing inflammation, and enhancing tissue repair.

1.8. Advantages of MSC-CM over cell-based therapy

MSC-CM offers several advantages over live MSC transplantation, including lower immunogenicity, reduced risk of tumor formation, easier storage and standardization, and improved safety profile. MSC-CM can be sterilized, lyophilized, and formulated as an “off-the-shelf” product, facilitating large-scale clinical translation.

Umbilical cord–derived MSCs, particularly WJ-MSCs, offer distinct advantages for secretome-based therapies due to

their non-invasive procurement, ethical acceptability, higher secretory activity, and reduced donor variability. Evidence from long-term expansion and molecular validation studies suggests that WJ-MSCs are especially suitable for generating standardized, reproducible MSC-conditioned media for regenerative and immunomodulatory applications.[9,10]

2. Challenges and Future Perspectives

Despite promising preclinical data, challenges remain in the clinical translation of MSC-CM. These include lack of standardized production protocols, batch-to-batch variability, optimal dosing, and delivery routes.[15] Future research should focus on defining potency assays, identifying key bioactive components, and conducting well-designed clinical trials to establish efficacy and safety.

Standardization of MSC-CM production must include defined culture conditions, passage restriction, senescence monitoring, secretome profiling, and validated potency assays to ensure reproducibility and regulatory compliance. Importantly, head-to-head comparative preclinical and clinical studies evaluating isolated MSCs, MSC-derived exosomes, and whole MSC-conditioned medium are necessary to determine relative efficacy, safety, scalability, and long-term therapeutic superiority. Such studies will be critical for guiding rational clinical adoption. .Advances in bioengineering, extracellular vesicle isolation, and regulatory frameworks are expected to further accelerate the development of MSC-CM–based therapeutics. **(Table 3)**

Table 3: Key challenges and proposed strategies in the development of cell-derived therapies

Challenge	Proposed strategies
Batch-to-batch variability	Standardized culture conditions, potency assays
Optimal dosing	Dose–response and pharmacokinetic studies
Delivery methods	Hydrogels, scaffolds, controlled-release systems
Regulatory approval	GMP-compliant production, quality contro
Mechanism validation	Identification of key bioactive components

3. Conclusion

The existing literature potentially supports the use of MSC-conditioned medium as a promising cell-free therapeutic strategy in regenerative medicine. Through its complex secretome, MSC-CM promotes angiogenesis, modulates immune responses, inhibits apoptosis, and activates endogenous repair mechanisms.

With rigorous standardization, potency validation, and well-designed comparative clinical trials, MSC-CM holds significant promise as a next-generation regenerative biologic.

4. Source of Funding

None.

5. Conflict of Interest

None.

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Cite this article: Mishra KK, Desai P, Pandey S, Padhiyar R, Khanna M. Mesenchymal Stem Cells (MSCs) conditioned medium for regenerative medicine: Mechanisms, applications, and translational perspectives. *Stem Cells Cell Ther Int.* 2026;1(1):1–4.