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# Conditioned medium from mesenchymal stem cells: Towards skin tissue engineering and wound healing

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**ABSTRACT**

Mesenchymal stem cells (MSCs) have emerged as a promising tool in regenerative medicine, particularly in skin tissue engineering and wound healing. Their paracrine activity, mediated by the secretion of bioactive molecules into conditioned medium (CM), has attracted significant attention as a cell-free therapeutic alternative. This review comprehensively examines the role of MSC-derived CM in promoting skin regeneration and wound repair. We discuss the key components of CM — growth factors, cytokines, extracellular vesicles, and miRNAs — that collectively modulate inflammation, angiogenesis, fibroblast proliferation, and extracellular matrix remodeling. Furthermore, we highlight the mechanisms by which CM enhances epithelialization, collagen synthesis, and scar reduction. Preclinical and clinical studies demonstrating the efficacy of MSC-CM in treating acute and chronic wounds, such as diabetic ulcers and burns, are critically evaluated. Challenges related to standardization, scalability, and regulatory approval are also addressed. This review underscores the potential of MSC-CM as a transformative approach in skin tissue engineering and wound healing, while calling for further research to optimize its therapeutic application.

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## Introduction

The field of skin tissue engineering has witnessed remarkable advancements in recent years, with stem cell-based therapies emerging as a promising strategy for enhancing tissue regeneration. Among these, mesenchymal stem cells (MSCs) have garnered significant attention due to their multipotent differentiation capabilities, immunomodulatory properties, and secretion of bioactive factors that promote tissue repair. A particularly innovative approach involves the use of MSC-conditioned media (CM), which contains a rich milieu of growth factors, cytokines, and extracellular vesicles (EVs) that mimic the regenerative effects of MSCs without the need for direct cell transplantation (1). This paradigm shift toward cell-free therapies addresses critical challenges, including immune rejection, ethical concerns, and scalability. In the context of skin tissue engineering, MSC-CM has demonstrated potential to accelerate wound healing, reduce inflammation, and promote angiogenesis and extracellular matrix remodeling (2). This review article aims to comprehensively explore the role of MSC-conditioned media in skin tissue engineering, highlighting its mechanisms of action, current applications, and prospects. By synthesizing recent findings and identifying key challenges, this work seeks to provide a foundation for further research and clinical translation in this rapidly evolving field.

### *Importance of skin tissue engineering in regenerative medicine*

Skin tissue engineering has emerged as a pivotal field in regenerative medicine, driven by the unique properties of the skin that make it an ideal candidate for innovative therapeutic strategies.

Its remarkable regenerative capacity, primarily mediated by resident stem cells and fibroblasts, allows for rapid repair of minor injuries (3). However, in cases of severe damage, such as chronic wounds, burns, or diabetic ulcers, this intrinsic healing process is often compromised, leading to prolonged inflammation, scarring, and functional impairment (4). Traditional approaches, including autographs and synthetic substitutes, are limited by issues such as donor site morbidity, immune rejection, and poor integration with host tissue. Skin tissue engineering overcomes these challenges by combining advanced biomaterials, bioactive molecules, and cellular therapies to create bioengineered constructs that mimic the skin's native structure and function. Stem cells, particularly MSCs, and dermal fibroblasts are central to this approach, as they promote extracellular matrix (ECM) remodeling, enhance angiogenesis, and modulate the inflammatory response (3). Recent innovations, such as 3D bioprinting and gene-edited cells, have further expanded the potential of engineered skin grafts to provide personalized, functional, and aesthetically superior solutions.

### *Overview of mesenchymal stromal cells (MSCs) and their therapeutic potential*

MSCs are widely recognized in regenerative medicine for

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their classic multilineage differentiation potential and their ability to sense and respond to microenvironmental cues (5). Recent studies have shown that MSCs can undergo metabolic reprogramming under stress conditions in damaged tissues, thereby enhancing their survival and therapeutic efficacy in tissue regeneration (5). Moreover, MSCs exhibit a unique ability to secrete trophic factors that orchestrate tissue repair through mechanisms such as mitochondrial transfer, which restores cellular bioenergetics in injured cells, and the release of exosomes packed with regenerative miRNAs and proteins (6). These advanced functionalities position MSCs not merely as passive participants but as active regulators of tissue homeostasis and repair.

In the context of tissue injury, MSCs have shown remarkable adaptability, particularly in modulating the immune prospect and promoting tissue regeneration without eliciting significant adverse effects. Emerging evidence highlights their role in epigenetic regulation, where they influence gene expression patterns in resident cells to foster a pro-regenerative environment (7). Li *et al.* showed that mice injected with MSCs overexpressing methyltransferase-like 3 (METTL3) and Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) increased miR-34a-5p, thereby reducing colorectal mucosal damage (8). The therapeutic efficacy of MSCs is primarily attributed to their paracrine activity, which involves the secretion of bioactive molecules such as growth factors, cytokines, and EVs (Figure 1). The factors present in MSCs' secretions collectively modulate key regenerative processes, including inflammation reduction, angiogenesis promotion, and extracellular matrix remodeling (9, 10). Accordingly, in the context of tissue injury, including skin, MSCs have demonstrated significant promise in addressing a wide range of conditions, from acute wounds to chronic degenerative diseases. Preclinical and clinical studies have highlighted their ability to enhance skin repair mechanisms, such as epithelialization, collagen synthesis, and scar reduction (11-13). Moreover, the advent of MSC-derived conditioned medium (CM) and exosomes has opened new avenues for cell-free therapies, minimizing risks associated with direct cell transplantation.

***Importance of mesenchymal stem cell-conditioned medium (MSC-CM) as a cell-free therapeutic approach***

Despite the remarkable potential of MSC therapy in regenerative medicine, several limitations hinder its widespread clinical application. Key challenges include low cell survival and engraftment rates post-transplantation, immune rejection risks, and the potential for uncontrolled differentiation or tumorigenicity (14). Kol *et al.* reported that intravenous injection of bone marrow MSCs (BM-MSCs) into healthy horses can elevate circulating CD8+ T cell levels after three injections (15). In another study, Huang *et al.* showed that intracardiac injection of allogeneic BM-MSCs into immunocompetent rats increased T cell and B cell activation and elevated antibody production against the allogeneic cells (16). Additionally, the heterogeneity of MSC populations and variability in donor sources can lead to inconsistent therapeutic outcomes. To address these issues, recent research has shifted focus to MSC-derived CM, which contains a rich array of bioactive molecules, including growth factors, cytokines, and EVs (Figure 2). MSC-CM refers to the cell culture supernatant collected after MSCs have been cultured in a defined medium for a specific period, during which they secrete a complex mixture of bioactive molecules (17). These molecules include growth factors (e.g., vascular endothelial growth factor [VEGF], transforming growth factor beta [TGF-β], epidermal growth factor [EGF]), cytokines, chemokines,

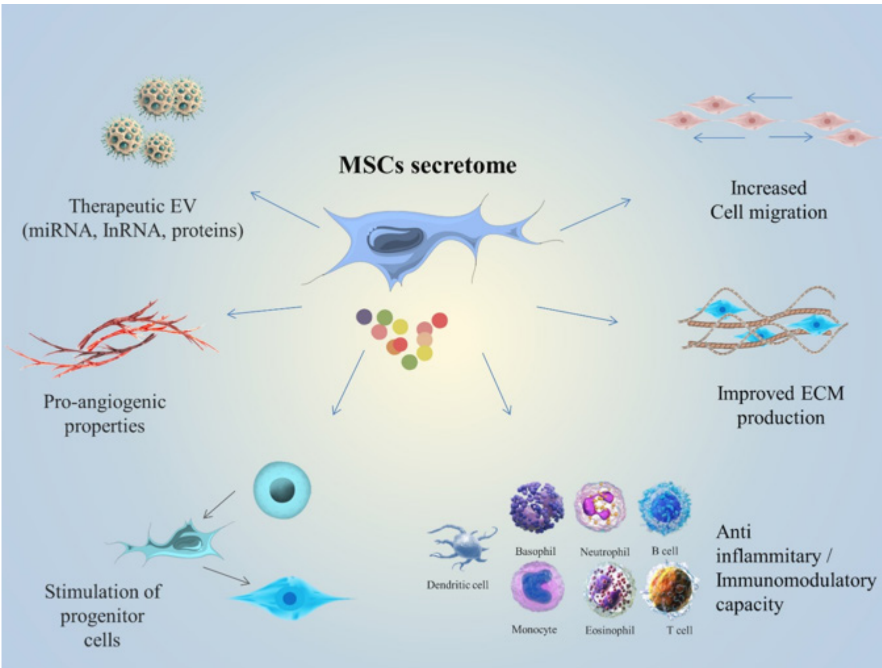


**Figure 1.** Schematic illustration of MSC-secreted factors driving tissue regeneration
The secretome stimulates EV-mediated therapeutic effects, accelerates angiogenesis, recruits endogenous progenitors, exerts anti-inflammatory and immunomodulatory effects, and improves ECM production, collectively improving tissue regeneration. MSC: Mesenchymal stem cells; EV: Extracellular vesicle; ECM: Extracellular matrix

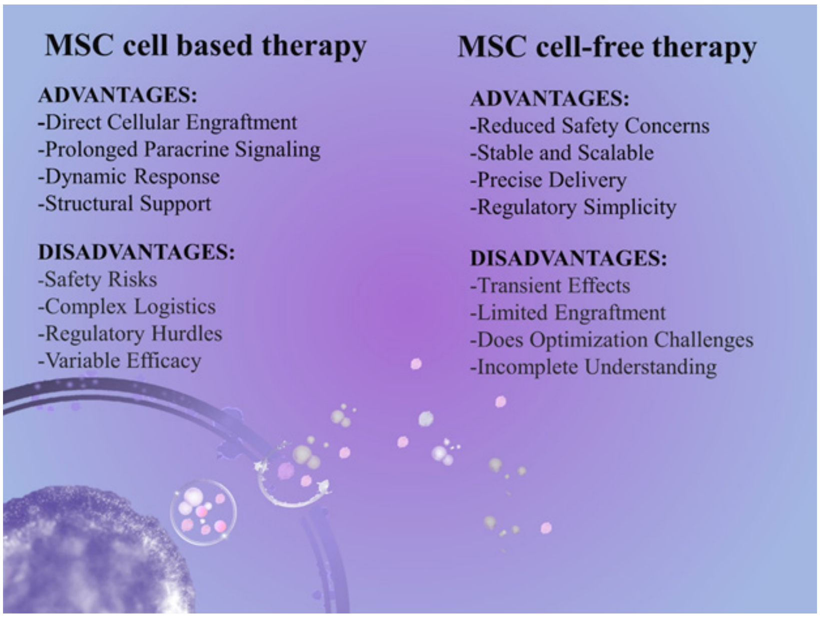


**Figure 2.** Comparative advantages and limitations of cell-based versus cell-free therapies using mesenchymal stem cells (MSCs)

EVs (including exosomes), and other paracrine factors that collectively contribute to tissue repair and regeneration. The composition of MSC-CM is highly dynamic and can be influenced by factors such as MSC source, culture conditions, and preconditioning strategies. This cell-free approach offers several advantages: it eliminates the risks associated with direct cell transplantation, provides a more standardized and scalable therapeutic product, and retains the paracrine effects of MSCs, which are now recognized as the primary mechanism behind their regenerative potential. The CM harnesses the anti-inflammatory, pro-angiogenic, and immunomodulatory properties of MSCs, making it a promising alternative for treating conditions such as chronic wounds, neurodegenerative diseases, and cardiovascular disorders (18). Furthermore, advances in bioengineering have enabled the optimization of CM production via techniques such as hypoxia preconditioning and 3D culture systems, thereby enhancing its therapeutic efficacy (19). By circumventing the limitations of traditional MSC therapy while preserving its benefits, CM offers a novel strategy that could redefine the future of regenerative medicine.

***Mesenchymal stem cell-conditioned medium: Composition and preparation scheme***

The composition of MSC-CM is highly complex and dynamic, encompassing a wide range of bioactive molecules that contribute to its therapeutic efficacy. These molecules include growth factors, cytokines, chemokines, EVs, and other paracrine factors, each playing a critical role in tissue repair and regeneration (19). Peshkova *et al.* showed that conditioned media collected from human bone marrow, adipose tissue, placenta, gingiva, and, primarily, umbilical cord MSCs secrete high levels of cytokines and growth factors, including EGF, PDGF, VEGF, IL-6, and IL-8, required for macrophage polarization and the suppression of inflammation (20). Below is a detailed breakdown of the key components typically found in MSC-CM and their approximate concentrations, where available:

***Growth factors***

Growth factors are proteins that regulate cellular processes such as proliferation, differentiation, and migration. They are among the most critical components of MSC-CM. Common growth factors and their typical concentration ranges include:

Vascular endothelial growth factor (VEGF): Promotes angiogenesis and endothelial cell survival. Concentrations in MSC-CM range from 100 to 500 pg/ml (21, 22).

Transforming growth factor-beta (TGF-β): Involved in extracellular matrix (ECM) production and immune modulation. Concentrations are typically around 1–10 pg/ml (23).

Epidermal growth factor (EGF): Stimulates epithelial cell proliferation and wound healing. Concentrations range from 50–200 pg/ml (23).

Fibroblast growth factor-2 (FGF-2): Promotes fibroblast proliferation and angiogenesis. Concentrations are usually 100–1500 pg/ml (23, 24).

Insulin-like growth factor-1 (IGF-1): Enhances cell growth and survival. Concentrations can be 1–5 ng/ml (24).

Platelet-Derived Growth Factor (PDGF): Stimulates cell migration and ECM production. Concentrations range from 100 to 2500 pg/ml (25).

***Cytokines and chemokines***

Cytokines and chemokines are small proteins that modulate immune responses and cell communication. Key examples include:

Interleukin-6 (IL-6): Plays a role in inflammation and tissue repair. Concentrations are typically 100–500 pg/ml (26, 27).

Interleukin-8 (IL-8): A chemokine that attracts neutrophils and promotes angiogenesis. Concentrations range from 500 to 1000 pg/ml.

Interleukin-10 (IL-10): An anti-inflammatory cytokine that reduces inflammation. Concentrations are usually 50–800 pg/ml(28).

Monocyte Chemoattractant Protein-1 (MCP-1/CCL2): Recruits monocytes and macrophages to sites of injury. Concentrations range from 5 to 50 ng/ml (29, 30).

***Extracellular vesicles (EVs)***

EVs, particularly exosomes, are membrane-bound vesicles that carry proteins, lipids, and nucleic acids (e.g., mRNA, miRNA) and play a significant role in cell-to-cell communication. The concentration of EVs in MSC-CM is typically measured in terms of particle count, often ranging from ~2.8 × 10^8 particles/10^6 cells (31). These vesicles contain:

***MiRNAs***

Such as miR-21, miR-29, and miR-146a, which regulate gene expression and promote tissue repair.

***Proteins***

Including heat shock proteins (HSPs), integrins, and tetraspanins, which contribute to cell signaling and ECM remodeling (32).

***Other proteins and bioactive molecules***

In addition to the proteins listed above, other bioactive molecules, such as hyaluronic acid, a glycosaminoglycan that supports ECM structure and hydration, are present in MSC-EV preparations (33), and their concentrations can vary widely depending on MSC source and the MSC-CM collection protocol.

Collagen and Fibronectin: These ECM proteins can provide structural support and promote cell adhesion. Their concentrations depend on the MSC source and culture conditions (34).

The composition and concentration of MSC-CM can vary depending on the MSC Source, culture conditions, and serum content. Bone marrow-derived MSCs may secrete higher levels of VEGF, while adipose-derived MSCs may produce more adipokines. Culture conditions such as hypoxia, 3D culture, or cytokine preconditioning can significantly alter the secretome profile. Additionally, serum-free or low-serum conditions are preferred to avoid contamination with exogenous proteins.

***Standard preparation and quantification of MSC-CM***

The standard preparation of MSC-CM involves a series of well-defined steps to ensure the quality and reproducibility of the final product (Figure 3). First, MSCs are isolated from their tissue source (e.g., bone marrow aspirate, adipose tissue, or umbilical cord) and expanded in culture using basal media such as Dulbecco's Modified Eagle Medium (DMEM) or Alpha-Minimum Essential Medium (α-MEM), supplemented with 10% fetal bovine serum (FBS) and antibiotics (e.g., penicillin-streptomycin) to support cell growth and prevent contamination. Once the cells reach 70–80% confluency, they are passaged to maintain their undifferentiated state and high viability. To prepare the conditioned media, MSCs are first washed thoroughly with phosphate-buffered saline (PBS) to remove any residual serum components that could interfere with downstream applications. The cells are then incubated in serum-free or low-serum media (e.g., containing 2% FBS or serum-free alternatives such as human platelet lysate) for a defined period, typically 24 to 72 hr. This incubation period allows MSCs to secrete their bioactive factors into the culture medium. The conditioning duration can be adjusted based on the desired concentration of secreted factors and the specific application (35).

To enhance the therapeutic potential of MSC-CM, preconditioning strategies are often employed. For instance, culturing MSCs under hypoxic conditions (1–5% $O_2$) can up-regulate the secretion of pro-angiogenic factors, while exposure to inflammatory cytokines like TNF-α or IFN-γ can enhance immunomodulatory properties. He *et al.* examined the efficacy of normoxic vs hypoxic cell culture conditions on the secretory profile of rat AD-MSC-CM

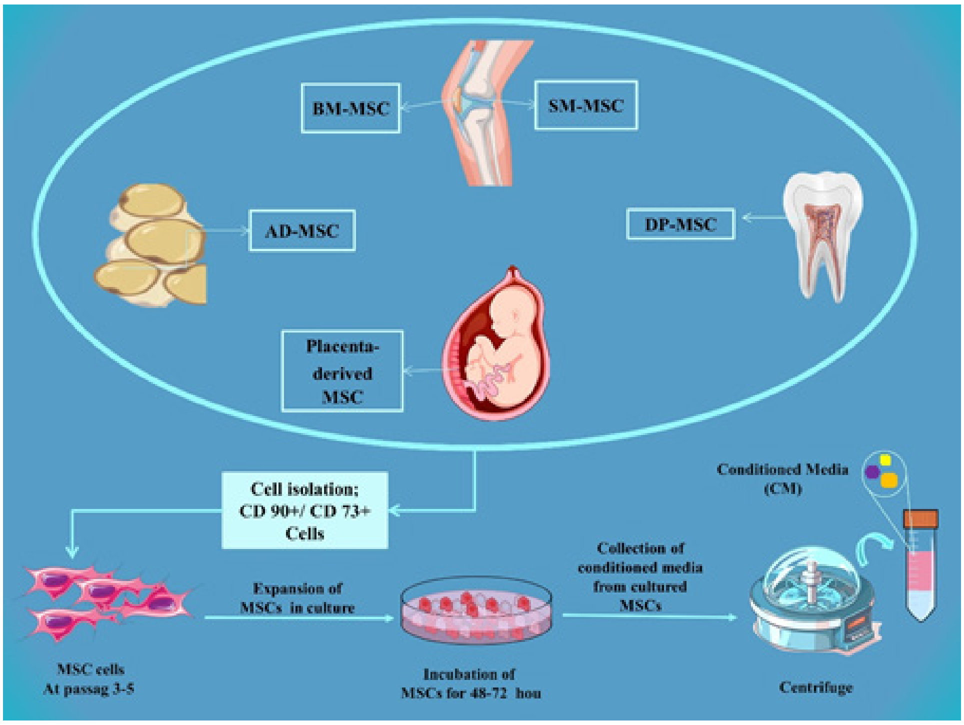


**Figure 3.** Schematic workflow of mesenchymal stem cell (MSC) isolation from diverse tissue sources and preparation of conditioned medium (CM)

AD-MSC: Adipose-derived mesenchymal stem cell, BM-MSC: Bone marrow-derived mesenchymal stem cell, SM-MSC: Synovial membrane-derived mesenchymal stem cell, DP-MSC: Dental pulp-derived mesenchymal stem cell.

using RT-PCR and ELISA, and also assessed the *in vivo* cardioprotective effects of hypoxic MSC-CM (hypoCM). They observed that hypoCM, compared with normoxic CM, expressed higher levels of hepatocyte growth factor (HGF), stromal-derived factor-1 (SDF-1), and VEGF. In addition, they observed that hypoCM reduced the infarcted muscle size and the cell apoptosis rate in the rat myocardial infarction (MI) model (36). Bulati *et al.* revealed that the immunosuppressive capacity of human ADMSCs can be promoted via "supportive signals" raised from interferon-γ (IFN-γ) priming of hAMSCs (γ-hAMSCs). Their results showed that γ-hAMSCs can promote an anti-inflammatory (M2)-like phenotype in monocytes and increase IL-10 levels, an anti-inflammatory cytokine (37).

Additionally, 3D culture systems, such as spheroids or scaffold-based cultures, have been shown to improve the quantity and quality of secreted factors compared to traditional 2D monolayer cultures.

After the incubation period, the conditioned media are collected and centrifuged at low speed (e.g., 300–500 × g) to remove cellular debris. The supernatant is then filtered through a 0.22 μm membrane to ensure sterility (19). Depending on the intended application, the MSC-CM can be used immediately, concentrated using ultrafiltration devices to increase the concentration of bioactive molecules, or lyophilized for long-term storage (38). Advanced techniques, such as proteomic analysis (e.g., ELISA and mass spectrometry), nanoparticle tracking analysis (NTA) for quantifying EVs, and RNA sequencing, for profiling miRNAs and other nucleic acids, are often employed to characterize the composition of the conditioned media, ensuring batch-to-batch consistency and identifying key factors responsible for its regenerative effects.

### *MSC-CM in wound healing: Preclinical and clinical evidence*

*In vitro* studies: Effects on keratinocytes, fibroblasts, and endothelial cells

*In vitro* studies demonstrate that MSC-CM enhances Keratinocyte/Fibroblast Proliferation. CM from umbilical cord MSCs (UC-MSCs) up-regulates epidermal growth factor (EGF) and fibroblast growth factor (FGF), accelerating scratch assay closure by 40–60% compared to controls (39, 40). Adipose-derived MSC-CM (AD-MSC-CM) can also ameliorate inflammation by reducing TNF-α and IL-6 secretion in macrophages by 50–70% via IL-10 and TGF-β pathways (41, 42).

It has been shown that MSC-CM can increase the angiogenic potential of fibroblasts through a 2-fold increase in capillary-like structures with bone marrow MSC-CM (BM-MSC-CM), linked to VEGF and angiopoietin-1 secretion (43).

### *In vivo animal models*

*In vivo* animal models are indispensable for evaluating the therapeutic potential of MSC-CM in chronic wound healing, as they recapitulate key pathophysiological features of human wounds, including impaired angiogenesis, persistent inflammation, and delayed re-epithelialization. Over the past decade, studies utilizing diabetic, ischemic, burn, and radiation-induced wound models in rodents (mice, rats), lagomorphs (rabbits), and large animals (pigs) have demonstrated MSC-CM's ability to accelerate closure, reduce fibrosis, and restore functional skin architecture (44, 45).

In a burn injury mouse model, 3D-spheroid MSC-CM obtained from bone marrow improved wound closure, with enhanced tube formation of endothelial cells *in vitro* mediated by increased chemokine (CXC motif) ligand 1 (CXCL1), Interleukin-6 (IL-6), IL-8, and vascular endothelial growth factor A (VEGFA) (46). Rat models treated with hypoxic human UC-MSC-CM showed improved fibroblast growth and collagen synthesis, and increased re-epithelialization, attributed to higher concentrations of wound-healing-related growth factors (47).

TNF-α stimulated Rat UC-MSC-CM loaded on topical gel in rat full-thickness skin defect increased the wound closure rate and fibroblast count in groups treated by MSC-CM loaded gel compared with control (48).

These models have also elucidated critical mechanistic insights, including the roles of exosomal miRNAs, cytokine networks, and scaffold-enhanced delivery, while highlighting translational challenges related to dosing, administration routes, and long-term safety. Table 1 presents the last 10 years of *in vivo* studies, followed by a discussion of innovative approaches and unresolved gaps in the field.

Despite promising preclinical results, key limitations hinder the translational potential of MSC-CM research in animal models. Species-specific differences in wound healing mechanisms (e.g., faster contraction in rodents vs humans) may overestimate therapeutic efficacy. At the same time, the lack of standardized protocols for CM preparation (e.g., variations in culture conditions, secretion profiles, and exosome isolation) complicates cross-study comparisons. Most studies focus on short-term outcomes (e.g., 14–28 days) (11, 44), with limited data on long-term safety, immune responses, or scar quality. Additionally, large-animal models (e.g., pigs and rabbits) remain underutilized despite their closer resemblance to human skin physiology. The absence of comorbid conditions (e.g., aging, multimicrobial infections) in many diabetic or burn models further reduces their clinical relevance.

To address the current challenges in preclinical MSC-CM research, several evidence-based strategies can enhance translational validity. First, standardization of CM production protocols—including defined culture conditions (e.g., hypoxia and 3D bioreactors), quantification of key bioactive factors (e.g., exosomes, VEGF, and TGF-β), and functional potency assays—is critical to ensure batch-to-batch consistency (19). Second, incorporating clinically relevant large-animal models (e.g., diabetic porcine wounds with impaired healing) can better mimic human pathophysiology and improve predictive value. Third, longitudinal studies assessing long-term safety (e.g., immunogenicity and tumorigenicity) and functional recovery (e.g., nerve regeneration and scar elasticity) are essential. Finally, integrating multi-omics approaches (single-cell RNA sequencing and proteomics) can identify conserved mechanisms across species and refine biomarker selection (56). Collaborative efforts to establish international guidelines for preclinical testing will accelerate the transition to clinical trials.

### *Clinical trials and translational applications of MSC-CM in wound healing*

The transition from promising preclinical results to clinical implementation of mesenchymal stem cell-conditioned medium (MSC-CM) for chronic wound management represents a critical juncture in regenerative medicine. As an infant field of study, clinical evidence in recent years has begun to validate the therapeutic potential of MSC-CM in addressing the complex pathophysiology of chronic wounds, particularly in diabetic foot ulcers, venous leg ulcers, and burn injuries. In this regard, two studies entered the clinical phase in 2023 (12, 57). Tan *et al.* used 10 % hUC-MSC-CM in a topical gel twice daily for 7 and 14 days in patients with diabetes mellitus and Hansen's Morbus (41 chronic ulcers) across four medical facilities. The study results showed a significant reduction in wound size (length, width, and area) (57).In the second study, Alinda *et al*. prepared AD-MSC-CM for the treatment of 32 patients with chronic plantar ulcers due to leprosy. Patients were randomised into two groups to receive either a framycetin gauze dressing only (n = 16) or topical AD-MSC-CM (n = 16), applied for up to 8 weeks (every 3 days). Their results showed that after 2 and 3 weeks from the start of treatments, there was a significant reduction in wound mean size and mean wound depth, respectively, in the AD-MSC-CM-treated group vs the framycetin gauze group (12).

These clinical investigations have demonstrated MSC-CM's capacity to modulate all phases of wound healing through its rich secretome of growth factors, cytokines, and extracellular vesicles, while overcoming many limitations associated with whole-cell therapies.

Current clinical trials have focused on three key therapeutic mechanisms: (1) angiogenesis promotion through VEGF and other pro-angiogenic factors, (2)

**Table 1.** *In vivo* preclinical studies of MSC-CM in chronic wound models (2015–2025)

| Study (Year) (Ref.) | Animal model | MSC source | Intervention | Fallow-up period | Main assessments | Key outcomes | Mechanistic insights |
|---|---|---|---|---|---|---|---|
| Wei *et al*. (2024) (45) | Immune-competent mouse wound model | Xenogeneic human ADMSCs | MSC-CM loaded on a composite biomatrix of a chondroitin sulfate/collagen scaffold (SC) coengrafted with MSCs | 7 days | Histochemical and immunohistochemical staining, RT–PCR | Significant increase in the xenogeneic human MSC survival after engraftment, immunomodulatory management of wound microenvironment | Increased level of inflammatory cytokines and macrophage polarization |
| Humenik *et al*. (2023) (44) | Dogs with complicated non-healing wounds | Canine AM-MSCs | Topical use of CM | 15 days | Scratch assay, macroscopic observation | Significant reduction in the wound surface area after 72 h post-treatment | Enhancement of the proliferative activity and migration of fibroblasts upon release of growth factors such as EGF, bFGF, and TGF |
| Zhang *et al*. (2020) (49) | Murine. Diabetic model. Full-thickness excisional skin wounds, | hUC-MSCs | Subcutaneously injected around the wounds | 14 days | Histological analysis and immunofluorescence analysis, RT–PCR | Promoted wound healing rate | Increased level of anti-inflammatory cytokines and VEGF promoted M2 macrophage polarization |
| Saheli *et al*. (2020) (50) | Mouse diabetic full-thickness excisional skin wounds model | Human BM-MSCs | Intraperitoneal injection of concentrated MSC-CM | 7 days | Histological analysis, RT-PCR, scratch assay | Accelerated wound closure | Higher cell migration and proliferation, up-regulated growth factors (bFGF, EGF) |
| Rong *et al*. (2019) (11) | Full-thickness skin punch in rats | ADMSCs | CM freeze-dried powder was mixed with the hydrogel | 16 days | Histological examination, ELISA detection, and real-time PCR | Faster wound healing in the rat treated with ASC-CM compared with the control | Significant increase in Col3A1/Col1A2, TGF-β3/TGF-β1, MMP1/TIMP1, and MMP3/TIMP1 in the ASC-CM group |
| Fridoni *et al*. (2019) (51) | Diabetic rats with Full thickness Skin wound | Human BM-MSCs | Intraperitoneal injection of concentrated MSC-CM combined with PBM | 4 days | Histological and stereological Studies, RT-PCR | Improved wound healing rate | Decreased neutrophils and macrophage numbers and increased fibroblast counts and improved angiogenesis process |
| Chen *et al*. (2018) (52) | Diabetic rat model with Full thickness | Human urine MSCs | Subcutaneous injection around the wounds | 12 days | Histological and immunofluorescence analysis | Increased wound closure rate | Promoted re-epithelialization, decreased scar |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Skin wound | | | | | | formation, and higher level of neoangiogenesis |
| Bagheri *et al.* (2018) (53) | Diabetic rat model with full thickness skin wound | Human bone marrow MSCs | Intraperitoneal injection of MSC-CM co-treated with PBM | 15 days | Histological and tensiometric examinations | Improved tensiometric properties | Immunomodulatory effects due to significant decrease in the total number of mast cells |
| Kouhk *et al.* (2018) (54) | Diabetic rat model with full thickness skin wound | Human bone marrow MSCs | Intraperitoneal injection of MSC-CM combined with PWLLLT | 15 days | Microbiological examinations, Tensiometric analysis | Faster wound closure | Improvement in biomechanical parameters of injured tissue |
| Ma *et al.* (2019) (55) | Diabetic rat model with Full thickness Skin wound | Human HF-MSC | Injection of MSC-CM into the wound | 12 days | Colony-forming efficiency, ELIZA detection, and histological assessments | Promoted wound healing | Higher epidermal thickness |

MSCs: Mesenchymal stromal cell; CM: Conditioned medium; ADMSCs: Adipose-derived mesenchymal stromal cells; hUC: Human umbilical cord; AM: Amniotic membrane; BM: bone marrow; PWLLLT: Pulsed wave low-level laser therapy; HF-MSC: Hair follicle MSCs; ASC: Antler stem cell; EGF: Epidermal growth factor; bFGF: Basic fibroblast growth factor; VEGF: Vascular endothelial growth factor; PBM: Photobiomodulation; Col: Collagen; TGF- β: Transforming growth factor beta; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitor of matrix metalloproteinase; HF: Hair follicle; RT-PCR: Reverse transcriptase-poly chain reaction

inflammation modulation via macrophage polarization and cytokine balance, and (3) extracellular matrix remodeling through controlled expression of MMPs and TIMPs (58, 59).

The cell-free nature of MSC-CM offers distinct advantages, including reduced immunogenicity, easier storage and administration, and potentially lower regulatory hurdles compared to live cell therapies (60). However, the field faces significant challenges in standardizing production protocols, characterizing active components, and optimizing delivery methods to ensure consistent clinical outcomes. Recent advances in extracellular vesicle characterization and proteomic profiling have enabled more precise quality control of MSC-CM preparations, addressing previous concerns about batch-to-batch variability. Furthermore, innovative delivery systems, such as hydrogels and spray formulations, have improved the pharmacokinetics of CM administration in clinical settings. This section critically evaluates the progress and limitations of clinical trials conducted between 2015 and 2025, providing insights into the evolving landscape of MSC-CM therapy for chronic wounds and outlining pathways for future clinical translation (Table 2).

**Table 2.** Recent clinical trials of mesenchymal stem cells-conditioned medium (MSCs-CM) in the treatment of chronic wounds registered in www.clinicaltrials.gov

| Study/NCT number | Phase | Sample volume | Fallow-up period | Intervention | Outcomes |
|---|---|---|---|---|---|
| Effectiveness of secretome from human umbilical cord mesenchymal stem cells in gel (10% SM-hUCMSC Gel) for chronic wounds (diabetic and trophic ulcer)–Phase 2 clinical trial /NCT04134676 (57) | Phase II RCT multicentral study | 41 chronic ulcer, patients with DFU and Hansen's Morbus | 2 weeks | Topical gel containing hUC-MSC-CM (10%) vs standard care, twice a day for two weeks | Significant decrease in mean ulcer length, width, and area |
| The efficacy of topical adipose mesenchymal stem cell-conditioned medium versus framycetin gauze dressing in chronic plantar ulcer of leprosy: A randomized controlled trial. 0052/LOE/302.4.2/VII/2020 (12) | Phase I RCT | 32 patients with chronic plantar ulcer of leprosy | 8 weeks | AD-MSC-CM applied as a topical treatment every three days | Reduction in both the size and depth of wounds by 82% and 95.8% and complete wound closure in some cases, without any adverse reaction |
| Mesenchymal stem cell conditioned medium-derived pleiotropic factor in treating residual burn wound/ NCT04235296 | Phase 1 | 30 | 2 months | Mesenchymal Stem Cell-derived pleiotropic factor in treating residual burn wound | NYR |
| Mesenchymal stem cell-derived pleiotropic factor in treating non-healing wounds/ NCT04235868 | Phase 1 | 30 | 1 month | Mesenchymal stem cell-derived bioactivator for treating chronic wounds | NYR |

RCT: Randomized clinical trial; DFU: Diabetic foot ulcer; hUC-MSC-CM: Human umbilical cord mesenchymal stem cell conditioned medium; AD-MSC-CM: Adipose-derived mesenchymal stem cell conditioned medium; NYR: Not yet reported

Collectively, these clinical trials demonstrate that MSC-CM therapy significantly enhances chronic wound healing across diverse etiologies (diabetic, venous, burn, and radiation-induced ulcers). Wound closure data showed higher complete closure rates in treated groups than in controls, with accelerated epithelialization (12).

Studies support that MSC-CM delivery via hydrogels and sprays improved CM retention, with fibrin dressings enhancing sustained release in burns (61).

In case of safety issues, no treatment-related serious adverse events (e.g., immunogenicity, tumorigenesis) have been reported, with transient mild erythema reported in <5% of cases (62). However, heterogeneity in CM potency (exosome content; particle count/ml across studies) and the lack of long-term recurrence data (>1 year) remain critical challenges (63).

### *Clinical challenges and future perspectives of mesenchymal stem cell-conditioned medium (MSC-CM) in skin wound repair*

Despite promising preclinical and early clinical results, translating MSC-CM into routine clinical practice faces several critical challenges. First, standardization issues plague CM production, with significant variability in secretome composition due to differences in cell sources (e.g., adipose vs bone marrow MSCs), culture conditions (e.g., hypoxia, 2D vs 3D systems), and isolation methods (e.g., exosome yield) (64). This heterogeneity leads to inconsistent therapeutic outcomes across trials. Second, the lack of defined potency assays complicates dose optimization and regulatory approval (65). While biomarkers such as VEGF and miR-21 correlate with efficacy, there is no consensus on quantifying "therapeutic units" of CM. Third, delivery limitations persist. Rapid clearance of liquid CM formulations often necessitates frequent applications, while emerging solutions (e.g., hydrogels and microneedles) require further validation in large-scale trials (66). Additionally, cost and scalability remain hurdles, as GMP-compliant CM production demands expensive infrastructure, particularly for exosome-enriched preparations.

It is expected that advancements in omics-guided quality control (e.g., proteomic fingerprinting of CM batches) and engineered exosomes (e.g., CRISPR-edited MSCs to enhance specific miRNAs) could improve reproducibility. Meanwhile, decentralized production models (e.g., automated bioreactors) may reduce costs. Clinically, phase III trials must prioritize patient stratification by wound etiology (e.g., diabetic vs venous) and long-term monitoring for recurrence and safety.

### *Advanced strategies for commercializing MSC-conditioned medium (MSC-CM): Key technological and regulatory considerations*

The commercialization of MSC-CM requires a multidisciplinary approach integrating bioprocessing optimization, standardized quality control, and regulatory compliance. First, scalable production must be achieved using bioreactor systems (e.g., 3D microcarriers or hollow-fiber bioreactors) to ensure consistent secretome yields while maintaining MSC potency (67). Second, lyophilization (freeze-drying) and nano-carrier encapsulation (e.g., liposomes or hydrogels) can enhance CM stability and shelf life (68). Third, potency assays must be developed to quantify critical bioactive components (e.g., exosomal miRNAs like miR-21 or VEGF concentrations) to meet FDA (Food and Drug Administration)/EMA (European Medicines Agency) guidelines. Finally, GMP-compliant manufacturing and Phase III clinical trials with stratified patient cohorts (e.g., chronic vs acute wounds) are essential for regulatory approval, leveraging lessons from ongoing MSC-derived exosome therapies.

## Conclusion

MSC-CM represents a transformative cell-free therapy for chronic wound healing, offering significant advantages over traditional cell-based approaches, including reduced immunogenicity, easier storage, and scalable production. Preclinical and clinical studies over the past decade have demonstrated its ability to modulate inflammation, promote angiogenesis, and enhance tissue regeneration through paracrine mechanisms. However, challenges such as secretome variability, lack of standardized potency assays, and delivery optimization must be addressed to ensure consistent clinical outcomes.

Prospectively, the field must prioritize GMP-compliant manufacturing, large-scale clinical trials, and regulatory harmonization to facilitate commercialization. Collaborative efforts between academia, industry, and regulatory bodies will be critical to establish guidelines for CM characterization and quality control. Future research should also explore personalized CM formulations tailored to specific wound etiologies (e.g., diabetic vs venous ulcers) and combination therapies with biomaterials or antimicrobial agents. By addressing these challenges, MSC-CM can transition from experimental studies to mainstream clinical practice, offering a safe and effective solution for chronic wounds worldwide.

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## Authors' Contributions

N N contributed to the conceptualization, methodology, and writing the original draft. P H drew schematic figures and contributed to editing the original draft. SM T served as the corresponding author, supervised the study, and contributed to the writing and review of the manuscript. All authors reviewed and approved the final version of the manuscript.

## Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this review article.

## Declaration

During the preparation of this work, the authors used DeepSeek Chat (an AI language model by DeepSeek) to assist with manuscript editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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