

Therapeutic Potential of Adipose-Derived Mesenchymal Stem Cell-Conditioned Medium in Inflammatory Bowel Disease

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Abstract:

Background: Mesenchymal stem cells (MSCs) are multipotent stromal cells with considerable therapeutic potential in regenerative medicine because of their ability to migrate toward sites of injury and exert immunomodulatory, angiogenic, and anti-apoptotic effects. These effects are largely mediated through paracrine signaling and the secretion of numerous bioactive factors. Adipose-derived mesenchymal stem cells (ADMSCs) are particularly promising because of their potent anti-inflammatory properties. However, conventional MSC-based cell therapy faces several limitations, including the need for extensive in vitro expansion, high costs, difficulties in cell delivery, reduced cell viability after transplantation, and potential genomic instability. Increasing evidence suggests that the therapeutic effects of MSCs are primarily mediated by their secreted paracrine factors rather than direct cellular engraftment or differentiation. These factors constitute mesenchymal stem cell-conditioned medium (MSC-CM), a complex mixture containing proteins, cytokines, growth factors, enzymes, extracellular vesicles, and coding and non-coding RNAs. MSC-CM may therefore provide a promising cell-free therapeutic approach with advantages related to storage, production, cost, and safety. It exerts immunomodulatory and regenerative effects by suppressing pro-inflammatory mediators, promoting anti-inflammatory cytokines, stimulating angiogenesis, and supporting tissue repair. Furthermore, ADMSC-conditioned medium has demonstrated beneficial effects in experimental inflammatory bowel disease by reducing colonic inflammation and modulating pro- and anti-inflammatory cytokines.

Keywords: Adipose-derived mesenchymal stem cells; Mesenchymal stem cell-conditioned medium; MSC-CM; Inflammatory bowel disease; Paracrine signaling; Immunomodulation; Regenerative medicine; Cytokines.

Introduction:

Mesenchymal stem cells (MSCs), a heterogeneous population of multipotent stromal cells, exhibit broad tissue distribution with established isolation protocols from bone marrow, adipose tissue, umbilical cord, and dental pulp (1). These cells have emerged as pivotal therapeutic agents in regenerative medicine due to their unique ability to migrate toward inflammatory sites and reparative mechanisms (2).

At injury microenvironments, MSCs engage in direct cellular crosstalk with immune cells (particularly T lymphocytes and macrophages) and secrete paracrine factors (e.g., PGE2, IDO, TGF- β) that collectively mediate immunomodulation and exert angiogenic and anti-apoptotic effects (3,4).

Mesenchymal stem cells (MSCs) can be isolated from diverse tissue sources, including bone marrow, adipose tissue, dental pulp, and umbilical cord, with each population exhibiting distinct secretory profiles that influence cellular migration. Comparative studies reveal that bone marrow stem cells (BMSCs) demonstrate the

highest mRNA expression of stromal-derived factor-1 (SDF1) and vascular cell adhesion molecule-1 (VCAM-1), along with elevated TNF- α levels, which may enhance hematopoietic stem cell homing but potentially exacerbate inflammatory responses in certain contexts (5). In contrast, priming of adipose tissue stem cells (ASCs) significantly upregulates immunomodulatory factors such as indoleamine 2,3-dioxygenase 1 (IDO1) and chemokine ligand 5 (CCL5), which contribute to their potent anti-inflammatory properties (6).

A 2019 meta-analysis of clinical trials demonstrated the therapeutic potential of mesenchymal stem cell (MSC) therapy for inflammatory bowel disease (IBD), showing favorable safety profiles and clinical efficacy in both Crohn's disease and ulcerative colitis (7).

Although MSCs are the most widely used stem cells, several challenges remain in cell therapy, including achieving stable cell passaging, creating laboratory conditions for stem cell culture, identifying the optimal mode of cell delivery, and a high-cost burden (8).

Therapeutic applications of stem cells require large-scale in vitro expansion to achieve clinically relevant cell numbers (typically 100-500 million cells per treatment). However, this expansion process introduces several significant challenges that can compromise therapeutic outcomes (9). During culture, stem cells frequently undergo metabolic and epigenetic changes that reduce their functionality upon transplantation (10). The transition from optimized culture conditions to the microenvironment of damaged tissues - characterized by hypoxia (often <1% O₂), nutrient deprivation, and acidic pH (6.5-6.8) - leads to rapid cell death, with studies reporting 40-70% viability loss within the first 72 hours post-transplantation (11).

A major concern is the high rate of apoptosis in transplanted cells, which not only reduces therapeutic efficacy but can also trigger adverse immune responses (12). Dying cells release damage-associated molecular patterns (DAMPs) that activate toll-like receptors on immune cells, potentially exacerbating inflammation at the injury site (13). Furthermore, extended in vitro culture increases genomic instability, with some studies reporting spontaneous transformation in 5-10% of mesenchymal stem cell lines after 15-20 subcultures. These transformed cells may exhibit tumorigenic potential if not properly screened before clinical use (14).

Limited sources and invasive procedures to harvest MSCs are key challenges of the use of MSC-CM. Recent studies suggest that the main driving force behind the therapeutic activity observed in mesenchymal stem cells (MSCs) are the paracrine factors secreted by these cells, generally referred to as MSC-conditioned medium. These biomolecules trigger antiapoptotic events to prevent further degeneration of the diseased organ through paracrine signaling mechanisms (15).

The rapid functional improvement observed within 72 hours post-stem cell transplantation suggests that therapeutic benefits are mediated primarily through paracrine mechanisms rather than cellular engraftment or differentiation. This early-phase recovery correlates with the secretion of a complex array of bioactive molecules that exert cytoprotective effects by inhibiting apoptotic pathways through mitochondrial stabilization (5,16).

The systemic distribution of paracrine factors through circulation enables both localized and distant therapeutic effects, including angiogenesis promotion and inflammation resolution (17).

The MSC-CM is a cocktail mixture of several hundreds to thousands of different proteins, cytokines, growth factors, and enzymes. MSC-CM also contains extracellular vesicles (EVs) as a cargo of various proteins, coding and non-coding RNA, small RNAs, autophagosomes, mitophagosomes (18).

Profiling of human embryonic stem cell-derived mesenchymal stem cell-conditioned medium (hESC-MSC-CM) identified 201 distinct gene products, including growth factors and cytokines implicated in critical biological processes such as cellular metabolism, immune defense, and tissue regeneration (5).

However, it is very difficult to claim the role of only a few growth factors or cytokines present in MSC-CM on tissue regeneration. Compared to cellbased therapies, CM may provide several advantages; it uses proteins rather than the whole cells to promote regeneration, and it could be stored for a long time without using any toxic reagent. The preparation of CM is more economical and it can be mass produced. The safety and efficacy evaluation of CM will be simpler, similar to traditional pharmaceutical preparations (18).

Mesenchymal stem cell-conditioned medium (MSC-CM) exerts potent immunomodulatory effects, primarily through secreted cytokines e.g., interleukin-10 (IL-10), transforming growth factor- β (TGF- β) and extracellular vesicles (EVs) that suppress pro-inflammatory mediators like TNF- α and IL-6 (19).

Vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in MSC-CM stimulate angiogenesis, restoring blood supply to damaged tissues. Simultaneously, matrix metalloproteinase inhibitors e.g., tissue inhibitor for metalloproteinase-1 (TIMP-1) in MSC-CM counteract collagen degradation, balancing ECM turnover to support scaffold stability for new tissue deposition (20).

The cytokines and growth factors in CM also play a key role in the chemotaxis of endogenous precursor cells. Chemotaxis of osteogenic and angiogenic precursor cells is essential for effective tissue regeneration (21).

Mesenchymal stem cell-conditioned medium (MSC-CM) could also promote the migration of endothelial cells and angiogenic differentiation (22). On the other hand, MSC-CM promotes macrophage chemotaxis (23).

The content of MSC-CM varies from cell type to culture condition. It is impossible to get the MSC-CM containing similar secretomes in each treatment in clinics. Therefore MSC-CM cannot guarantee a similar effect in every treatment. Absence of the worldwide protocol for MSC-CM harvesting for tissue regeneration application is also a problem. So far, there is no data to illustrate that CM from a specific MSC origin is suitable for the specific tissue regeneration and identifying optimal delivery routes either systemic infusion or localized application to maximize therapeutic efficacy (24).

A number of studies have demonstrated therapeutic impacts of adipose-derived mesenchymal stem cells (ADMSCs) and MSC-conditioned medium (MSC-CM) on different inflammatory diseases, such as acute kidney injury, pulmonary hypertension, arthritis, experimental autoimmune encephalomyelitis, and diabetes (25).

Regarding to therapeutic roles of ADMSCs and MSC-CM in IBD progression, they could be among the best candidates in developing alternative therapies for the patients. Studies demonstrate that ADMSCs-CM significantly reduces colonic inflammation in murine colitis models by suppressing pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β) while upregulating anti-inflammatory mediators (IL-10, TGF- β) (26).

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