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In Vitro Transcription as a Strategy to Enhance Mesenchymal Stem Cell Secretome for Therapeutic Use: An Overview

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ABSTRACT

Mesenchymal stem cells (MSCs) have become a cornerstone of regenerative medicine owing to their capacity to secrete a diverse array of bioactive molecules, collectively termed the secretome. The MSC secretome exerts profound immunomodulatory, anti-inflammatory, and trophic effects that underpin much of the therapeutic efficacy observed in MSC-based interventions. Nevertheless, variability in the secretory profile across donors, tissue sources, and culture conditions continues to limit the reproducibility and potency of MSC-derived therapies. Recent advancements in in vitro transcription (IVT) mRNA technology have emerged as a robust and transient platform for the reprogramming of mesenchymal stem cells (MSCs) without the need for genomic integration. Through the use of IVT-mRNA-mediated expression of selected cytokines, growth factors, or homing receptors, MSCs can be endowed with enhanced anti-inflammatory and regenerative capabilities while preserving their native phenotype and viability. This review summarizes current IVT-mRNA–based strategies for engineering the MSC secretome, with an emphasis on augmenting anti-inflammatory cytokines (e.g., IL-10, TSG-6) and growth factors (e.g., VEGF, HGF, FGF2). The review also examines how IVT-mRNA redefines the cellular secretory landscape, outlines key considerations in IVT-mRNA design and optimization, and discusses translational implications for both cell-based and cell-free therapeutic applications. Finally, it underscores persistent challenges, including transient transgene expression, innate immune activation, and delivery inefficiency, and contemplates future prospects for integrating IVT-mRNA technology with advanced biomaterials, cellular priming methodologies, and multifactorial modulation to achieve consistent and potent therapeutic secretomes.

Keywords: Mesenchymal stem cells (MSCs), secretome, In Vitro Transcription (IVT); mature mRNA, regenerative medicine cell therapy

1. Introduction

Mesenchymal stem cells (MSCs) are a type of progenitor cell that has the capacity to differentiate into various lineages[1], including osteogenic,chondrogenic, and adipogenic. However, their primary function in tissue repair and immunomodulation is attributed to their ability to secrete factors that regulate other cells in the surrounding tissue [2,3]. In contrast to the direct engraftment or differentiation of stem cells, the therapeutic effects of mesenchymal stem cells (MSCs) are predominantly attributable to their secretome, a complex mixture of soluble cytokines, growth factors, chemokines, lipids, and extracellular vesicles (EVs)[4]. The secretome in question has been demonstrated to play essential roles in modulating immune cell activity, attenuating inflammation, promoting angiogenesis, and supporting tissue regeneration in a variety of disease contexts. These contexts include, but are not limited to, autoimmune disorders, ischemic injury, and fibrosis[5–7]. Despite

promising results in preclinical and clinical studies, several barriers limit the reproducibility and potency of MSC-based therapies. The MSC secretome is highly sensitive to donor variability, tissue source, passage number, and culture conditions. Furthermore, once transplanted, MSCs frequently encounter harsh microenvironments (hypoxia, oxidative stress, inflammation) that diminish their survival and secretory capacity. Consequently, there is growing interest in strategies to engineer the MSC secretome toward a more defined, stable, and therapeutically potent composition.

Conventional methods employed to augment the MSC secretome encompass preconditioning (e.g., exposure to hypoxia, inflammatory cytokines, or pharmacological agents), genetic modification via viral vectors, and the selection of specific MSC subpopulations[8]. While these methods have provided important insights, they each have significant limitations. Viral vectors are characterized by their ability to facilitate stable transgene expression; however, they are also associated with the potential for insertional mutagenesis and prolonged ectopic expression[9]. Despite their apparent simplicity, preconditioning methods yield transient and frequently heterogeneous effects. Therefore, an alternative approach is necessary that will allow for the control of protein expression in a transient, non-integrative, and tunable manner.

Messenger RNA (mRNA)-based technology, including in vitro transcription (IVT), has recently emerged as a powerful platform for achieving these objectives [10,11]. The process of IVT-mRNA facilitates the transient expression of specific genes by introducing synthetic mRNA transcripts that are capped and polyadenylated, and which are introduced directly into cells. In contrast to plasmid or viral vectors, IVT-mRNA functions within the cytoplasm, circumventing the requirement for nuclear entry and evading genomic integration. Furthermore, chemical modifications of nucleotides, such as the incorporation of pseudouridine (Ψ) or 1-methylpseudouridine (m1Ψ)[12], have been shown to significantly reduce innate immune sensing and improve translation efficiency. These advances have led to widespread adoption of IVT-mRNA in immunotherapy and vaccination, and are now being extended to regenerative medicine and stem cell engineering. Applied to MSCs, IVT-mRNA provides a versatile and controllable means to enhance or reprogram secretory functions. Transient overexpression of cytokines such as interleukin-10 (IL-10)[13–15] or tumor necrosis factor-stimulated gene-6 (TSG-6)[16] can potentiate anti-inflammatory effects, while expression of growth factors such as vascular endothelial growth factor (VEGF)[17] or hepatocyte growth factor (HGF)[18–20] can enhance regenerative capacity and angiogenesis. In addition, it is possible to design IVT-mRNA to encode receptors such as CXCR4 or PSGL-1. This has the potential to improve MSC homing to sites of injury or inflammation. Consequently, this could optimize the delivery of therapeutic secretomes *in vivo*.

The objective of this review is to provide a concise overview of the recent advancements in IVT-mRNA strategies for enhancing the MSC secretome. In this discussion, the principles of IVT-mRNA design and delivery are examined, with particular attention to examples of successful MSC engineering for cytokine and growth factor secretion. Additionally, the cellular mechanisms that mediate secretome modulation are explored. A particular focus is placed on methodological innovations, including but not limited to: nucleotide modification, untranslated region (UTR) optimization, and delivery vector design. These innovations play a pivotal role in determining the stability, translational efficiency, and immunogenicity of in vitro-transcribed mRNA. In conclusion, the current challenges, safety considerations, and future directions for integrating IVT-mRNA engineering with advanced biomaterials and multi-factor modulation to produce potent, reproducible, and clinically translatable MSC secretomes are highlighted.

2. Mesenchymal Stem Cell Secretome: Composition and Therapeutic Significance

The mesenchymal stem cell (MSC) secretome encompasses the full repertoire of bioactive molecules secreted into the extracellular milieu, including soluble proteins, lipids, nucleic acids, and extracellular vesicles (EVs). This complex secretory output constitutes the primary mechanism by which MSCs exert therapeutic effects in vivo, shifting the paradigm from cell replacement toward paracrine-mediated repair. Numerous studies now demonstrate that the regenerative and immunomodulatory benefits of MSC transplantation can be replicated by the conditioned medium or purified secretome alone, underscoring the importance of understanding and engineering its composition.

2.1. Composition of the MSC secretome

The MSC secretome is comprised of two predominant fractions: The first category is composed of soluble factors, including cytokines, chemokines, and growth factors. The second category consists of vesicular components, such as microvesicles and exosome. These vesicular components encapsulate proteins, lipids, messenger ribonucleic acids (mRNAs), and microRNAs[20]. The soluble fraction contains a range of anti-inflammatory cytokines (e.g., IL-10, TGF-β, PGE2), angiogenic factors (VEGF, FGF2, PDGF), and trophic molecules that promote cell survival and tissue remodeling (HGF, IGF-1). The vesicular fraction, particularly the exosome, plays a crucial role in intercellular communication by facilitating the transfer of regulatory molecules. These molecules have the capacity to modulate immune cell polarization, endothelial activation, and tissue regeneration. The relative abundance and bioactivity of these components are influenced by a complex interplay of intrinsic and extrinsic factors. A variety of factors have been demonstrated to influence the qualitative and quantitative profile of the secretome. These factors include donor age, tissue source (bone marrow, adipose tissue, umbilical cord, or dental pulp), culture conditions, oxygen tension, and exposure to inflammatory stimuli[21,22]. The inherent variability of MSC-based therapies poses a significant challenge to the reproducibility and standardization of these treatments.

2.2. Therapeutic functions of the MSC secretome

The MSC secretome has been demonstrated to elicit pleiotropic therapeutic effects by modulating a variety of cellular processes:

a. Immunomodulation and anti-inflammation: MSC-derived factors, including IL-10, TSG-6, prostaglandin E2 (PGE2), and indoleamine 2,3-dioxygenase (IDO), have been shown to suppress the activation of pro-inflammatory immune cells, such as Th1 and Th17 cells, and M1 macrophages, while promoting regulatory T cells and M2 macrophages. This shift has been demonstrated to contribute to the resolution of chronic inflammation and the protection of host tissues[23–25].
b. The process of angiogenesis, along with that of tissue regeneration, is of particular interest in this study. Secreted growth factors, including VEGF, HGF, and FGF2, have been shown to stimulate endothelial proliferation, migration, and capillary formation. The angiogenic effects described herein are of critical importance in ischemic tissues, wherein vascular repair is a prerequisite for regeneration[26].
c. The effects of the substance in question include anti-apoptotic and cytoprotective properties. The secretome has been demonstrated to enhance cell survival under stress conditions through paracrine activation of pro-survival pathways, such as PI3K/Akt and ERK1/2. It has been demonstrated that factors such as HGF and IGF-1 play a crucial role in the protection of host cells from apoptosis and oxidative damage[27].
d. Fibrosis attenuation and remodeling: MSC-derived molecules, including matrix metalloproteinases (MMPs) and transforming growth factor-beta (TGF-β) modulators, play a

crucial role in extracellular matrix remodeling. This process is pivotal in limiting fibrosis while promoting functional recovery in damaged tissues[28,29].

Together, these effects have been demonstrated in diverse preclinical models including myocardial infarction, liver injury, lung fibrosis, spinal cord injury, and autoimmune diseases. It is important to note that MSC-conditioned medium or purified secretome preparations can reproduce many of these benefits without the need for live-cell transplantation. This development paves the way for cell-free therapies.

2.3. Limitations and rationale for secretome engineering

Despite these advantages, the therapeutic performance of MSC secretome is inconsistent. Batch-to-batch variability, short in vivo half-life, and inadequate secretion of key bioactive molecules often limit efficacy [30,31]. Furthermore, the natural secretome reflects the physiological state of MSCs, which may not be optimal for therapeutic applications. For instance, mesenchymal stem cells (MSCs) cultured under normoxic conditions or in standard media secrete relatively low levels of interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF), reducing their anti-inflammatory or regenerative potency.

Table 1. Comparison of genetic and non-genetic strategies for modulating MSCs secretomes.

Strategy	Type	Integration risk	Duration of expression	Example factors	Major advantages	Limitations
Viral vectors	Genetic	High	Long-term	IL-10, HGF	High efficiency	Risk of insertional mutagenesis
Plasmid DNA	Genetic	Moderate	Moderate	VEGF, FGF2	Simple design	Low efficiency in MSCs
IVT-mRNA	Non-genetic	None	Transient (24-72 h)	IL-10, VEGF, TSG-6	High safety, tunable, rapid and high expression	Limited duration
Protein priming	Non-genetic	None	Transient	HGF, IL-6	Simple application	Limited control, cost
Hypoxia preconditioning	Non-genetic	None	Transient	VEGF, IL-6	Mimics physiological condition	Variable reproducibility (inconsistent)

In order to address the aforementioned limitations, researchers have developed several strategies for modulating the MSC secretome. These strategies include preconditioning (hypoxia, cytokine stimulation), genetic modification, and biomaterial-based culture systems[32]. Among these, in vitro transcription (IVT)-mRNA-mediated engineering represents a particularly promising approach[11] (Table 1). The transient expression of selected genes without genomic integration enables the precise modulation of anti-inflammatory cytokines or growth factors through the use of IVT-mRNA, effectively "programming" MSCs towards a therapeutically advantageous secretory phenotype[10,11]. Thus, the next frontier in MSC-based therapy lies in the rational design and

modulation of the secretome itself, transforming MSCs into bioactive delivery platforms capable of producing tailored therapeutic factors on demand.

3. Mechanism and Principle of IVT-mRNA Engineering in MSCs

In vitro transcribed (IVT) mRNA engineering in mesenchymal stem cells (MSCs) is a method for introducing specific genetic instructions to alter the cells' function temporarily without modifying their genome. This approach utilizes synthetic messenger RNA molecules to instruct MSCs to produce the desired proteins, a process that relies on the natural cellular machinery for translation. The mechanism involves two primary stages: the in vitro synthesis of engineered mRNA and the subsequent delivery and translation of this mRNA within the MSCs[33].

3.1 Fundamentals of IVT-mRNA technology and its structural components

In vitro transcription (IVT), derived messenger RNA (mRNA) technology represents a cell engineering platform that enables transient, non-integrative expression of target proteins. In this approach, synthetic DNA templates containing the gene of interest downstream of a bacteriophage promoter (typically T7, SP6, or T3) are transcribed *in vitro* using RNA polymerase. The resulting mRNA transcript can be chemically or enzymatically modified to mimic the structure of mature eukaryotic mRNA, thereby enhancing its translational efficiency and stability in mammalian cells[34,35].

An IVT-mRNA molecule typically consists of five main structural components (Table 2): a 5’ cap, 5’ untranslated region (UTR), open reading frame (ORF), 3’ UTR, and a poly(A) tail. Each component plays a crucial role in determining the stability, translational efficiency, and immunogenicity of the transcript. The 5’ cap structure (commonly the anti-reverse cap analog, ARCA, or the more advanced CleanCap™) protects the mRNA from exonuclease degradation and facilitates ribosome recruitment. The 5’ UTR influences translation initiation and can be engineered with optimized Kozak sequences or untranslated motifs from highly expressed genes to enhance translation. The ORF encodes the protein of interest, typically an anti-inflammatory cytokine (e.g., IL-10, TSG-6) or a trophic growth factor (e.g., VEGF, HGF, FGF2), optimized for codon usage compatible with human MSCs to maximize protein yield. The 3’ UTR and poly(A) tail regulate transcript stability and translation. Tail length (usually 100-150 adenosines) is critical; too short leads to rapid degradation, while excessively long tails may reduce translation efficiency. 3’ UTR motifs from stable transcripts such as β-globin or α-globin mRNAs are frequently adopted to prolong half-life[36].

3.2 Chemical modifications and optimization

One of the most transformative steps in IVT technology has been the incorporation of modified nucleosides to minimize innate immune activation. Substituting uridine with pseudouridine (Ψ) or 1-methylpseudouridine (m1Ψ), and cytidine with 5-methylcytidine (m5C)[12], markedly reduces recognition by pattern recognition receptors such as TLR3, TLR7, TLR8, and RIG-I. These modifications enhance both transcript stability and translation in MSCs. Enzymatic or co-transcriptional capping ensures proper orientation and high capping efficiency, while rigorous purification using high-performance liquid chromatography (HPLC) or cellulose-based methods removes double-stranded RNA contaminants that otherwise trigger antiviral responses.

3.3 Cellular uptake of mature mRNA and its translation in MSCs

Efficient mRNA delivery into MSCs is essential for transient but robust expression. Following transfection, IVT-mRNA is internalized into the cytoplasm of MSCs through lipid nanoparticle encapsulation, cationic polymers, or electroporation. Electroporation achieves high transfection

efficiency but may transiently affect cell proliferation; hence, optimization of pulse voltage and duration is essential for maintaining MSC characteristics[37]. Unlike DNA-based vectors, IVT-mRNA bypasses nuclear entry, thus circumventing barriers related to nuclear membrane permeability and cell-cycle dependence. Once inside the cytoplasm, the 5'-cap structure of IVT-mRNA recruits the eukaryotic initiation factor (*eIF4E*) complex, which initiates ribosome binding and protein translation. The newly synthesized protein undergoes post-translational modification and is secreted through the endoplasmic reticulum (ER)-Golgi pathway, contributing directly to the MSC secretome. In MSCs, the efficiency of mRNA translation is influenced by factors such as cell passage, metabolic activity, and the intracellular abundance of RNA-binding proteins. Studies have demonstrated that MSCs exhibit a favorable translation environment for IVT-mRNA, with sustained protein production for 24–72 hours post-transfection without compromising cell viability, phenotype, or differentiation potential[34,36]. This transient expression window is particularly advantageous for regenerative applications requiring controlled, short-term release of therapeutic proteins.

3.4 Immunological considerations and cellular stress responses

One of the major challenges in IVT-mRNA applications is the potential activation of innate immune pathways triggered by double-stranded RNA contaminants or unmodified nucleotides[38]. In MSCs, such immune activation can lead to the induction of type I interferon responses, upregulation of stress-related genes, and suppression of translation. To mitigate this, purification methods such as high-performance liquid chromatography (HPLC) or cellulose-based extraction are employed to remove double-stranded RNA species. Furthermore, the incorporation of modified nucleotides and the optimization of UTRs have been demonstrated to reduce immune activation, thereby preserving the homeostasis and viability of MSCs.

3.5 Advantages of IVT-mRNA over DNA-based engineering

Compared to viral or plasmid-based systems, IVT-mRNA engineering offers several key advantages for MSC modification[11]:

a. Safety: No genomic integration or insertional mutagenesis.
b. Efficiency: Direct cytoplasmic translation independent of nuclear transport.
c. Temporal Control: Expression duration can be tuned via sequence design and formulation.
d. Scalability: Rapid synthesis and standardized quality control enable reproducible production.
e. Flexibility: Easy co-transfection of multiple mRNAs to enable synergistic effects (e.g., co-expression of IL-10 and VEGF).

The combination of these characteristics establishes IVT-mRNA as a remarkably versatile and clinically adaptable approach for the customization of the MSC secretome towards specific therapeutic objectives.

4. Strategies for Enhancing the MSC Secretome Using IVT-mRNA

Mesenchymal stem cells (MSCs) primarily exert their therapeutic effects through the paracrine action of their secretome, which comprises soluble factors and extracellular vesicles enriched with bioactive proteins and nucleic acids. The composition of this secretome determines the magnitude and type of biological response in recipient tissues, ranging from anti-inflammatory modulation to pro-regenerative signaling. Engineering the MSC secretome using in vitro transcribed (IVT) mRNA provides a highly controllable, transient, and non-integrative method to augment specific cytokines and growth, thereby enhancing therapeutic efficacy[34,37]. In contrast to stable genetic modification via viral vectors, IVT-mRNA induces transient expression lasting 24-72 hours, which is sufficient for the secretion of therapeutic proteins without resulting in permanent genomic

alteration. This transient reprogramming facilitates the secretion of elevated levels of anti-inflammatory cytokines or growth factors by MSCs while minimizing safety concerns (Table 3). By designing polycistronic or co-transfection systems, multiple secretome-related genes (e.g., IL-10 + HGF + CXCR4) can be simultaneously expressed, leading to synergistic enhancement of immunomodulatory and regenerative functions.

This section summarizes IVT-mRNA–based strategies for modulating key functional components of the MSC secretome, focusing on (i) anti-inflammatory cytokines, (ii) pro-regenerative growth factors, and (iii) combinatorial or multiplexed modulation for synergistic effects.

4.1 IVT-mRNA–mediated expression of anti-inflammatory cytokines

Inflammation control is a critical determinant of tissue recovery and immune balance in regenerative therapies. MSCs naturally secrete anti-inflammatory mediators such as interleukin-10 (IL-10), transforming growth factor-β (TGF-β), and tumor necrosis factor–stimulated gene 6 (TSG-6). However, their basal levels are often insufficient to counteract excessive inflammation in pathological contexts. IVT-mRNA technology has been employed to transiently overexpress these cytokines, effectively amplifying MSC immunomodulatory capacity without permanent genetic alteration.

IL-10 mRNA modification in MSCs[13–15], for example, has been shown to significantly reduce pro-inflammatory cytokine production (IL-1β, IL-6, TNF-α) by macrophages in co-culture systems. The increased IL-10 secretion also enhances the polarization of macrophages toward an M2 phenotype, promoting tissue repair and reducing fibrosis. Similarly, TSG-6 mRNA transfection enhances MSC-mediated suppression of neutrophil infiltration and decreases inflammatory lesion size in preclinical models. Importantly, these modifications do not alter MSC surface markers (CD73, CD90, CD105) or their differentiation potential, confirming the safety and phenotypic stability of IVT-mRNA–engineered cells.

Moreover, the kinetic control provided by IVT-mRNA expression allows temporal adjustment of cytokine release. This is particularly useful in therapeutic contexts requiring a short burst of anti-inflammatory signaling, such as acute tissue injury or early post-transplant inflammation, followed by natural resolution of immune activation.

4.2 IVT-mRNA–driven enhancement of regenerative growth factors

Growth factors play pivotal roles in angiogenesis, tissue remodeling, and cellular proliferation, processes central to MSC-based regenerative therapy. IVT-mRNA engineering can augment the production of such factors in MSCs, thereby enhancing their paracrine regenerative effects. For instance, VEGF (vascular endothelial growth factor) mRNA transfection significantly promotes endothelial tube formation and neovascularization in ischemic tissues. Similarly, HGF (hepatocyte growth factor) and FGF2 (fibroblast growth factor 2) mRNA delivery enhances cell migration, survival, and extracellular matrix remodeling. Importantly, the transient expression achieved through IVT-mRNA ensures a self-limiting release profile, which mitigates risks associated with uncontrolled angiogenesis or fibrotic signaling that are occasionally observed with viral-based overexpression systems. In addition to single-factor modulation, multi-mRNA delivery enables simultaneous upregulation of synergistic proteins. For example, co-delivery of VEGF and HGF mRNAs has been shown to result in additive angiogenic and cytoprotective effects compared to single-gene expression. This modularity provides an efficient means of tailoring MSC secretome profiles to target specific regenerative pathways.

4.3 Multiplexed and combinatorial modulation of MSC secretome

The therapeutic outcome of MSC secretomes often depends on a coordinated network of signals rather than a single dominant factor. IVT-mRNA technology allows the design of multiplexed transfection strategies that co-express several cytokines and growth factors to mimic this complex biological interplay. For instance, simultaneous overexpression of IL-10, CXCR4, and SIRT1 has been proposed to enhance both the anti-inflammatory and homing capabilities of MSCs, as well as to improve their resistance to oxidative stress. Such combinations can be optimized by balancing expression kinetics, codon usage, and mRNA stoichiometry to avoid translational competition. Furthermore, incorporating mRNA sequences encoding transcriptional regulators, such as NRF2 (a master regulator of antioxidant responses), may indirectly modulate multiple downstream secretome components, offering a systems-level approach to MSC enhancement.

4.4 Integration with preconditioning and biomaterial systems

The performance of IVT-mRNA–engineered MSCs can be further augmented through integration with physical or biochemical preconditioning methods. Hypoxic culture, for example, naturally enhances MSC secretion of VEGF, IGF-1, and IL-8; combining this with targeted IVT-mRNA delivery yields additive or synergistic effects. Additionally, encapsulating IVT-mRNA–transfected MSCs in biomaterial scaffolds or hydrogels can sustain their viability and control the release kinetics of secreted proteins[39]. This hybrid approach enables localized, prolonged therapeutic effects while maintaining the transient, non-integrative nature of the mRNA modification.

Table 3. Examples of IVT-mRNA-based secretome enhancement in MCSs

Target protein	Functional category	Observed effect	Expression duration	Application model
IL-10	Anti-inflammatory cytokin	M2 macrophage polarization, reduced TNF-α	48-72 h	In vitro inflammation model
TSG-6	Anti-inflammatory mediator	Reduced neutrophil infiltration	24-48 h	Murine wound model
VEGF	Angiogenic factor	Enhanced tube formation, neovascularization	48 h	Ischemic limb model
HGF	Growth factor	Increased cell survival, anti-apoptotic effect	24-72 h	Myocardial injury model
FGF2	Mitogenic factor	Improved proliferation	48 h	Wound healing model
IL-10 + VEGF	Dual cytokine/growth factor	Additive anti-inflammatory and pro-angiogenic response	48 h	Co-culture/ischemic model

5. Future Directions and Concluding Remarks

The application of in vitro transcription (IVT)-mRNA to engineer the mesenchymal stem cell (MSC) secretome represents a transformative convergence of synthetic biology and regenerative medicine. Although significant progress has been made in optimizing mRNA chemistry, purification, and delivery, further innovation is required to fully realize its potential for clinical translation.

5.1 Toward multi-gene and modular secretome engineering

Future IVT strategies will likely shift toward multi-factorial reprogramming, where synthetic mRNA cocktails encode combinations of anti-inflammatory cytokines (e.g., IL-10, TSG-6), trophic factors (e.g., VEGF, HGF, FGF2), and chemotactic molecules (e.g., CXCR4). By transiently coordinating multiple signaling pathways, MSCs can be tuned for specific therapeutic niches such as ischemic tissue repair, neuroinflammation, or fibrosis resolution. Modular design frameworks, employing standardized UTRs and codon-optimized ORFs, may enable scalable and customizable secretome "profiles" tailored to different pathophysiological contexts.

5.2 Integrating IVT-mRNA with biomaterials and exosome platforms

Combining IVT-engineered MSCs with smart biomaterials, such as injectable hydrogels, extracellular matrix scaffolds, or nanoparticle reservoirs, could allow localized and sustained release of secreted bioactive factors[40]. Moreover, mRNA-engineered MSCs may serve as factories for therapeutic exosomes enriched in desired proteins or microRNAs, extending the functional impact of transient mRNA expression beyond the parent cells. Future work should aim to map the proteomic and transcriptomic landscape of IVT-modified secretomes to define optimal expression kinetics and cargo distribution in extracellular vesicles.

5.3 Overcoming technical and regulatory challenges

Despite its promise, IVT-based MSC engineering faces several hurdles[41]:

a. Transient expression: While this approach may offer certain benefits in terms of safety, it does impose limitations on the long-term effects. One potential solution to this challenge could involve the implementation of strategies that involve repeated dosing or the co-delivery of the substance with slow-release systems.
b. Innate immune activation: Continued refinement in nucleoside modification, purification, and carrier formulation is imperative to minimize unwanted interferon responses.
c. The third component pertains to the manufacturing scalability and reproducibility. The development of standardized, GMP-compatible workflows for the production, purification, and MSC transfection of IVT-mRNA will be pivotal for clinical translation.

From a regulatory perspective, IVT-mRNA–engineered MSCs are classified as a hybrid category between gene therapy and cell therapy products. It is therefore imperative that clear guidance on safety testing, stability, and potency assays be established to facilitate the advancement of these products toward clinical approval.

6. Conclusion

IVT-mRNA technology offers a highly flexible, transient, and safe platform for reprogramming the MSC secretome. Its ability to enhance the secretion of anti-inflammatory cytokines and regenerative growth factors, without altering the genome, addresses many of the limitations of traditional genetic modification approaches. As mRNA design continues to evolve,

integrating it with biomaterials, omics-guided secretome profiling, and precision delivery systems will enable next-generation MSC therapies with predictable potency and minimal risk. Ultimately, IVT-mRNA–engineered MSCs and their secretomes represent a paradigm shift in regenerative medicine, moving from cell replacement toward cell-derived, programmable biotherapeutics capable of orchestrating complex tissue repair and immune modulation.

Competing Interests

The authors declare no competing interests.

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