# Recent Advances and Future Perspectives in Large-Scale Production and Isolation of hMSC-Derived Exosomes: Emphasizing the Role of 3D Wave Bioreactor Technology

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# Abstract

# Human mesenchymal stem cells (hMSCs) possess tremendous therapeutic potential for a wide range of diseases due to their self-renewal, multi-lineage differentiation capacity, and immunomodulatory effects. Exosomes secreted by hMSCs not only inherit these beneficial properties but also exhibit lower immunogenicity and superior safety profiles, rendering them ideal candidates for cell-free therapies. However, the clinical translation of hMSC-derived exosomes remains hindered by several challenges, such as limited cell culture density, suboptimal exosome yield, and the lack of efficient large-scale isolation technologies. Thus, establishing robust and scalable production and purification processes for hMSC-derived exosomes is crucial. Recent advances have addressed many of these obstacles. This review comprehensively summarizes the latest progress in elucidating the biogenesis of hMSC-derived exosomes, optimizing large-scale culture conditions, and developing novel isolation strategies. In addition, we briefly discuss the biomedical and therapeutic applications of hMSC exosomes. Notably, the advent of three-dimensional (3D) large-scale culture systems has overcome the limitations in cell density and batch variability associated with traditional two-dimensional (2D) cultures. This review further compares the effects of different bioreactor systems, particularly glass stirred tanks and wave bioreactors, on hMSC proliferation and exosome production. Emerging evidence suggests that wave bioreactor systems offer substantial advantages in supporting cell expansion and boosting exosome yield. In summary, these innovations not only provide a cutting-edge strategy for exosome manufacturing but also lay a solid foundation for the clinical translation of hMSC-derived exosome therapies.

**Keywords：**hMSC-derived exosomes; Large-scale production; 3D wave bioreactor; Isolation techniques; Cell-free therapy; Bioprocess optimization

# 1. Introduction

Human mesenchymal stem cells (hMSCs) are a type of stem cells that can be isolated from adult tissues and have the ability to self-renew and multidirectional differentiation [1]. With the continuous deepening of relevant research, hMSCs have gradually been applied to the fields of tissue repair, immune regulation and disease treatment. The effects of hMSCs are mainly attributed to two mechanisms: cell differentiation into target cells for integration and repair; and regulation of the behavior of other cells by paracrine signals [1-2]. Human mesenchymal stem cells (hMSCs) are widely recognized for their regenerative and immunomodulatory capabilities, positioning them as a valuable tool for advanced cell-based therapies [3]. However, it is now well-established that much of their therapeutic efficacy is mediated not by direct cell replacement but by paracrine mechanisms-particularly through the release of extracellular vehicles (EVs), with exosomes being the most biologically active subset [4]. hMSC-derived exosomes, 40 ~ 150 nm vesicles enriched in functional proteins, lipids, and RNAs, have emerged as a promising cell-free therapeutic alternative due to their excellent safety profile, low immunogenicity, and ability to cross biological barriers [5].

Despite growing interest, the clinical translation of exosome-based therapies remains hindered by production bottlenecks. Traditional two-dimensional (2D) culture systems, commonly used for hMSC expansion, offer limited surface area, low cell density, and inconsistent exosome yields [6-7]. These limitations do not meet the quantity and quality requirements necessary for clinical-grade exosome manufacturing [8]. More importantly, the functional quality of exosomes is influenced by culture conditions, making standardization critical for ensuring product efficacy and safety [9].

To address these challenges, considerable effort has been made to establish three-dimensional (3D) culture platforms that support higher cell densities and enhanced extracellular vesicle output [10]. Unlike static 2D monolayers, 3D systems better replicate the native stem cell microenvironment, providing improved nutrient gradients, oxygenation, and mechanical cues. Among these, bioreactor-based culture strategies have demonstrated strong potential for scalable, controlled hMSC culture [11-12]. Stirred-tank bioreactors (STBs) were initially adopted due to their prevalence in industrial bioprocessing [13]. However, the high shear stress and turbulent mixing associated with STBs can damage both hMSCs and exosomes, resulting in poor viability and reduced functional exosome recovery [14].

A significant breakthrough in the field has been the application of wave bioreactors (WBRs) for hMSC culture and exosome production [15]. WBRs utilize a gentle rocking motion to promote fluid mixing, thereby minimizing shear stress while ensuring the effective delivery of oxygen and nutrients [16]. This system facilitates suspension culture within single-use bags, offering a closed, scalable, and GMP-compliant environment ideal for therapeutic manufacturing [17]. Recent studies have shown that hMSCs cultured in WBRs not only demonstrate higher viability and proliferation rates compared to those grown in stirred-tank systems, but they also secrete significantly greater yields of exosomes with preserved bioactivity [18-20].

The WBR system also supports key advantages over conventional approaches: it allows high-density cell growth in a dynamic yet low-shear microenvironment, reduces batch-to-batch variation, and is compatible with automation and downstream integration [21-23]. Moreover, the system offers a reproducible platform for long-term cultures that require minimal manual intervention, which is essential for clinical-grade production pipelines [24]. Alongside advancements in culture systems, improvements in exosome isolation and purification technologies such as tangential flow filtration and size exclusion chromatography have further enabled the establishment of large-scale workflows [25-27]. However, the selection of an appropriate culture platform remains a critical determinant of both the productivity and biological quality of exosomes [28].

Functionally, hMSC-derived exosomes have demonstrated therapeutic efficacy across a broad spectrum of diseases [29]. In cardiovascular disorders, they promote angiogenesis and reduce fibrosis; in neurological diseases, they modulate inflammation and enhance synaptic repair; and in renal fibrosis, they inhibit profibrotic pathways such as TGF-β/Smad and activate AMPK signaling [30-31]. The finding from various disease models underscores the capacity of exosomes to modulate cellular pathways in a manner that is comparable to, or potentially exceeds, the efficacy of their parent cells [32].

In conclusion, hMSC-derived exosomes present significant potential for next-generation regenerative and immunomodulatory therapies. However, achieving scalable and reproducible production remains a critical barrier. The development and application of 3D bioreactor systems, especially the wave-induced bioreactor, represent a transformative advancement in exosome manufacturing. These bioreactors create an advantageous biomechanical and metabolic environment conducive to the expansion of hMSCs and the secretion of exosomes, thereby offering a practical pathway for clinical translation. Continued optimization and integration of upstream culture and downstream purification processes will be essential for realizing the full therapeutic potential of hMSC-derived exosomes. Establishing a robust and scalable production and isolation process for hMSC-derived exosomes is of utmost importance. Thus, this review provides a comprehensive overview of the recent advancements in exosome biogenesis, optimization of large-scale culture conditions, and various separation techniques.

## 2. Characterization of Exosomes derived from different hMSCs

### 2.1 Exosomes derived from different hMSCs

The existing classifications of stem cells comprise embryonic stem cells, induced pluripotent stem cells, and adult stem cells. Among the various types of adult stem cells, human mesenchymal stem cells (hMSCs) are a particularly important type of adult stem cell. hMSCs originally referred to bone marrow stromal fibroblasts isolated from adult bone marrow with osteogenic effects [33]. However, subsequent studies have found that almost all adult tissues contain hMSCs [34]. The currently recognized minimum standard for hMSCs was proposed by ISCT in 2006 [35]. 1) MSCs can adhere to plastic surfaces; 2) MSCs must express CD105, CD73, and CD90, and lack the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR; 3) MSCs must be able to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro. The unique characteristics of hMSCs bring unique functions. Endogenous asymmetric division endows MSCs with continuous self-renewal ability [36]; The lack of expression of HLA-DR and co-stimulatory molecules involved in T and B lymphocyte activation (such as CD80, CD86 and CD40) leads to low immunogenicity [37]; At the same time, hMSCs have multidirectional differentiation potential and are non-tumorigenic. Therefore, MSCs are safe and effective in treating diseases such as tissue repair [38] and immune regulation [39].

MSCs can be divided into two major sources: neonatal tissue and adult tissue. Neonatal tissue-derived hMSCs are the youngest and most primitive hMSCs without invasive acquisition procedure, such as human amniotic mesenchymal stem cells (hAMSCs) [40]; human umbilical cord blood mesenchymal stem cells (hCBMSCs) and human umbilical cord mesenchymal stem cells (hUC-MSCs) [40-41]. Bone marrow mesenchymal stem cells (hBMSCs) and adipose tissue-derived mesenchymal stem cells (hADSCs) isolated from adult tissues have been extensively studied [42-44]. In addition, hMSCs can also be isolated from various organs, such as dental pulp mesenchymal stem cells (hDPSCs) and skin mesenchymal stem cells (hSMSCs) [45-46]. hMSCs from different sources differ in cell characteristics, functions, and isolation methods. The specific information of several common types of hMSCs is summarized (**Table 1**). For MSCs, the “drug” for treating disease is the cells themselves [47]. Exosomes secreted by hMSCs can also play the same role as hMSCs and perform cell-free therapy [48]. With lower immunogenicity, higher safety and simpler storage methods, they have considerable application prospects in disease treatment.

### 2.2 Characterization and biogenesis of exosomes

Exosomes are characterized as a subtype of extracellular vesicles that are secreted by cells through a process known as budding. Typically, the diameter of conventional exosomes ranges from approximately 40 to 150 nm [49-51]. However, some studies have found that the size ranges of apoptotic exosomes, autophagic exosomes, and nanoparticles overlap with those of exosomes, as shown in **Figure 1A**. Therefore, exosomes require additional evaluation criteria [51]. **Table 2** provides examples of extracellular vesicles or non-vesicle components in the range of 40~150 nm.

The prototype of the exosome biogenesis model comes from the study of reticulocyte vesicles [52]. First, the plasma membrane invaginates. In this process, some substances in the extracellular environment are internalized into the cell and wrapped by the phospholipid bilayer to form primary vesicles. Primary vesicles fuse with each other to form early endosomes (EE). Early endosomes may fuse and communicate with some intracellular membrane systems, such as mitochondria, endoplasmic reticulum, and Golgi apparatus [53]. The early endosomes then mature into late endosomes (LE). Late endosomes undergo multiple invaginations to form intraluminal vesicles (ILV), which are future exosomes. During the late endosome invagination process, many substances in the cytoplasm enter the vesicles, such as metabolites, nucleic acids, proteins and lipids [54]. The intraluminal vesicles eventually develop into multivesicular bodies (MVB). Proteins expressed on the surface of MVB determine the fate of MVB. Some MVBs fuse with autophagosomes or lysosomes and are degraded; some MVBs are transported to the plasma membrane and fuse with the plasma membrane to release ILV as exosomes [53]. The complexity of the generation process determines the richness of exosome components and the diversity of their functions [55]. Exosomes are nanoscale particles that contain limited substances [56]. Moreover, different types of cells, different microenvironments in which cells are located, and different physiological responses of cells will affect the secretion and material composition of exosomes [57]. Therefore, exosomes are heterogeneous and the composition and biogenesis of exosomes are shown in **Figure 1**.

Inferred from the generation process, exosomes mainly contain phospholipids, proteins, nucleic acids and metabolites. These material bases determine the characterization methods and application characteristics of exosomes [58]. The phospholipid bilayer of exosomes inherits the permeability and fluidity of the cell membrane, which facilitates the exosomes to shuttle between tissues and cells to transmit information. Therefore, exosomes are of great application value in drug loading. For example, Elnaz et al. [57] loaded doxorubicin (DOX) into purified mouse bone marrow mesenchymal stem cell-derived exosomes by electroporation, achieving safe and multifunctional delivery of DOX to colon adenocarcinoma. Some proteins are highly enriched on the exosome membrane (such as CD63, CD81 and CD9) and can be used for Western blot analysis (WB) to identify the presence of exosomes and conduct relative quantitative analysis; exosome surface proteins may also mediate many pathological and physiological processes through signal transduction. For example, Fas expressed on the surface of NK-Exos binds to FasL on tumor cells, triggering the extrinsic apoptosis pathway by activating caspase-8/3 and PARP30, thereby inducing cell apoptosis [58]. Nucleic acids within the lumen of exosomes can play unexpected roles by regulating gene expression in target cells.

In order to further meet the current clinical application needs of high-yield, high-quality, and high-purity exosomes, bioreactor-scale culture of hMSCs has become a rapid and efficient application method.

## 3. 3D culture technology for production of hMSCs -exosomes

### 3.1 2D and 3D culture for production of hMSCs -exosomes

The production efficiency and biological quality of hMSC-derived exosomes are profoundly influenced by the cell culture system [59]. Traditional 2D monolayer cultures, while widely used, offer a limited surface area and an artificial, static microenvironment, which restrict cell–cell and cell–matrix interactions [60]. This results in low cell density, reduced viability, and suboptimal exosome yield and bioactivity [61]. In contrast, 3D culture systems—such as spheroids, microcarrier-based suspension cultures, and especially bioreactor-based platforms—provide a more physiologically relevant environment [62]. These systems enable natural cell interactions and improved diffusion of nutrients and oxygen, resulting in sustained stemness, higher cell densities, and significantly greater exosome output. Notably, exosomes produced in 3D cultures have been reported to possess enhanced cargo composition, superior regenerative and immunomodulatory bioactivity, and greater homogeneity compared to those from 2D cultures (**Table 3**).

### 3.2 3D Wave Bioreactor Culture Mode and Its Technical Advantages

Among 3D culture technologies, the wave bioreactor has emerged as an advanced and scalable platform for hMSC expansion and exosome production [63]. The wave bioreactor operates by gently rocking a disposable culture bag, which contains hMSCs attached to microcarriers or growing as aggregates [64]. This rocking motion ensures efficient mixing and oxygenation with minimal shear stress, preserving both cell viability and exosome integrity [65-66]. The dynamic 3D environment better mimics physiological conditions, enhances cell–cell and cell–matrix communication, and supports higher cell densities than static systems.

Technically, the wave bioreactor allows precise real-time control of critical culture parameters, such as pH, dissolved oxygen, and temperature, using integrated sensors and process analytical technology (PAT) [67-69]. Feeding can be managed through fed-batch or perfusion strategies to extend culture duration and maximize cumulative exosome yield [70-72]. Importantly, serum-free or chemically defined media are often adopted to avoid contamination from exogenous exosomes and to simplify downstream purification [75-76].

### 3.3 Comparative Advantages over Stirred-Tank Bioreactors and Process Optimization

Direct comparison with stirred-tank bioreactor systems highlights the intrinsic advantages of the wave bioreactor for hMSC-exosome manufacturing(**Figure 2**). Stirred-tank bioreactors, characterized by high shear forces due to mechanical agitation, frequently compromise cell viability, disrupt microcarrier integrity, and negatively impact exosome yield and bioactivity [77-80]. In contrast, the wave bioreactor's low-shear environment better preserves the hMSC phenotype, supports higher-quality and more uniform exosome output, and facilitates large-scale, GMP-compliant production. Superior oxygen and nutrient distribution ensure homogeneous cell growth, while the closed system design further reduces contamination risk and enhances operational safety [81-86].

Furthermore, the wave bioreactor is highly adaptable to process intensification strategies such as hypoxic preconditioning, cytokine or small molecule supplementation, and mechanical cue modulation, all of which can further amplify exosome biogenesis and therapeutic efficacy [87-88]. This coordinated engineering and bioprocess optimization underscores the wave bioreactor's position as the gold standard for industrial-scale, high-quality exosome production (**Table 4**).

**3.4 Strategies for Increased exosome secretion**

During exosome formation, from cellular endocytosis to the release of multivesicular bodies (MVBs), a variety of intracellular substances are closely related to the production of exosomes [89-90]. The fate of MVBs also directly determines whether exosomes are secreted. There are three key steps in the secretion of MVB: targeted transport, docking with the plasma membrane, and fusion with the plasma membrane [53,91]. Among them, cytoskeletal proteins-actin filaments and microtubules coordinate and drive the targeted transport and exocytosis of MVBs [92]. Rab GTPase regulates the docking and fusion of MVBs with the plasma membrane, promoting the transport and secretion of exosomes [93]. The core protein complex (SNARE) ultimately mediates the fusion of MVBs with the cell membrane [94]. In addition, tumor susceptibility gene 101 protein (TSG101), apoptosis-inducing factor 6 interacting protein (ALIX), syndecan-1, endosomal sorting and transport complex (ESCRT), phospholipids, tetracaines, ceramide and sphingomyelinase may be involved in the origin and biogenesis of exosomes [53]. Therefore, the regulation of stem cell exosome secretion should be based on the substance and pathway regulation of the exosome generation process. At present, there are many strategies to increase the secretion of exosomes, including physical signal stimulation, molecular interference, culture environment factor stimulation, and external inducer stimulation [95]. Some specific methods to increase the secretion of exosomes are listed (**Table 5**). These methods can be combined with bioreactor to propose strategies for large-scale production of exosomes from hMSCs.

**3.5 Outlook: Toward Clinical-Grade Exosome Manufacturing**

The transition from 2D to 3D anchored by the wave bioreactor platform has established a new benchmark for exosome manufacturing, enabling both scalability and product excellence. Exosomes produced under these conditions consistently exhibit enhanced cargo profiles, superior regenerative and immunomodulatory function, and robust batch uniformity, as validated in diverse preclinical models [79,80,95]. The convergence of advanced bioprocess engineering, real-time analytics, and microenvironmental modulation positions the wave bioreactor as the enabling technology for the clinical and commercial realization of hMSC-exosome therapeutics [96-99].

## 4. Extraction of Exosomes from hMSCs

The rapid advancement of exosome-based therapeutics and drug delivery has driven the urgent need for efficient, high-purity, and scalable exosome isolation technologies [101]. The selection and optimization of exosome separation methods are fundamentally dictated by the physical and chemical properties of exosomes, including size, density, solubility, and surface biomolecular specificity [102].

### 4.1 Isolation methods based on exosome size

*4.1.1 Ultracentrifugation*

Ultracentrifugation (UC) separates exosomes based on their size and density, as shown in **Figure 3A**. UC remains the gold standard for exosome isolation, relying on sequential centrifugation steps to separate exosomes based on size and density2. While UC is mature and low-cost, it is labor-intensive, time-consuming, and often results in relatively low purity and recovery [103-105].

*4.1.2 Density gradient ultracentrifugation*

Density gradient ultracentrifugation (DGUC) is a separation method based on ultracentrifugation using two or more separation media of different densities, as shown in **Figure 3B**. DGUC employs separation media of varying densities to achieve higher purity exosome fractions [106-107]. Although improved in selectivity, DGUC is operationally complex and unsuitable for large-scale applications.

*4.1.3 Ultrafiltration*

In ultrafiltration (UF), the acquisition of exosomes relies on filter membranes with different pore sizes or molecular weight cutoffs, as shown in **Figure 3C**. UF utilizes membranes with defined pore sizes or molecular weight cutoffs to retain exosomes while allowing smaller molecules to pass [106]. Tangential flow filtration (TFF) overcomes clogging issues seen in dead-end filtration and is more amenable to scale-up [108-109]. UF is simple and efficient, but membrane fouling and sample loss can affect yield.

*4.1.4 Size exclusion chromatography*

In size exclusion chromatography (SEC), biological fluids serve as the mobile phase and porous materials serve as the stationary phase [101], as shown in **Figure 3D**. SEC separates exosomes based on their ability to enter porous stationary phases [101,110]. SEC delivers high-purity exosomes and preserves biological activity. It is often used in combination with UF or UC to enhance purity and yield [111]..

### 4.2 Isolation methods based on exosome solubility

Polymer precipitation (PBP) refers to the fact that after adding certain polymers, the solubility of exosomes decreases and they precipitate, as shown in **Figure 3E**. By adding polymers like PEG, exosome solubility decreases, enabling precipitation at low-speed centrifugation [112-114]. PBP is rapid and user-friendly but may introduce contaminants and residual toxicity, limiting its application in downstream uses [115].

### 4.3 Isolation methods based on exosome protein specificity

Immunoisolation (IA) utilizes antibodies against exosome surface proteins (e.g., CD9, CD63, CD81) immobilized on carriers such as magnetic beads or chromatography column [116-118]. Depending on the different antibody carriers, it can also be divided into immunomagnetic bead method and column chromatography method, as shown in **Figure 3F/G**. In addition, IA is often combined with microfluidics to separate exosomes with high purity. For example, Panwar et al. [119] conjugated specific antibodies for tetracaines on the surface of exosomes to gold nanoparticles and established an effective exosome isolation strategy. IA offers outstanding specificity and purity but is limited by high material costs and scalability challenges.

### 4.4 Isolation methods based on exosome lipid specificity

Certain metal oxides (e.g., TiO₂, ZrO₂) and molecules like TIM4 or polycationic polymers can selectively bind exosome membrane lipids or charge, enabling rapid isolation (**Figure 3H)**. Based on this principle, a study attempted to specifically separate exosomes by micron-sized TiO2 particles [120]. As a result, the model exosomes achieved a 93.4% separation recovery rate within 5 minutes. Lipid binding and protein binding can also be combined. Zhang et al. [121] synthesized a new exosome separation material Fe3O4@TiO2-CD63. Using this material, 92.6% of intact exosomes were separated within 10 minutes. Yoshida et al. [122] allowed TIM4 to bind to the exosome membrane component phosphatidylserine (PS) in a Ca2+-dependent manner. Using the above magnetic beads, high purity and high yield of exosomes were isolated within 40 minutes[123]. Hybrid strategies combining lipid and protein targeting enhance yield and specificity.

### 4.5 Microfluidics

Microfluidics (MF) is a technology that can be used to separate and detect exosomes from small amounts of samples with high precision in microfluidic devices[124] (**Figure 3I**). Microfluidics technology is usually based on a combination of multiple principles. Numerous studies have shown that microfluidics technology is efficient and precise, but microfluidic chips are too complex and have low processing throughput [125-127]. Thus, integrating multiple methods is a future trend. Notably, 3D cultures yield higher concentration and purity of exosomes than 2D, while ultrafiltration, though efficient, still faces industrial scalability challenges [127]. The specific information on the above exosome isolation methods is summarized (**Table 6**).

**4.6 Outlook and Future Directions**

Each isolation method presents distinct advantages and limitations in terms of yield, purity, scalability, and operational complexity7. Increasing evidence supports the combined use of multiple methods to synergistically improve exosome recovery and function [125-127]. However, the field still lacks standardized, high-throughput, cost-effective solutions compatible with clinical and industrial-scale needs8.

Notably, 3D mass-cultured hMSCs consistently produce exosomes at higher concentrations and with more complete morphology compared to equivalent 2D cultures **(Figure 3S**). The exosomes extracted from 3D cultures demonstrate not only greater abundance but also enhanced purity and integrity, underscoring the superiority of 3D culture systems for exosome production.

## 5. Application of Exosomes in Biomedicine and Disease

With the rapid development of 3D dynamic culture systems, exosomes derived from hMSCs cultured under 3D conditions have demonstrated superior yield, purity, and functional activity compared to 2D culture, making them a focus of current translational and clinical research. Here, we highlight recent studies where exosomes were explicitly generated by 3D culture platforms, summarizing their preparation methods, disease applications, and underlying mechanisms.

**5.1 Autoimmune and Inflammatory Diseases**

Rheumatoid Arthritis: Exosomes from hMSCs cultured in 3D hollow-fiber bioreactors (TFF/SEC purified) significantly suppressed synovial inflammation, promoted M2 macrophage polarization, and ameliorated joint damage in CIA mouse models, outperforming 2D-exosome controls (Zhang et al., Adv Sci, 2020).

Colitis: 3D-cultured hUCMSC-exosomes, produced in microcarrier-based stirred-tank bioreactors and isolated by TFF+anion exchange chromatography, restored epithelial barrier function and reduced inflammation more effectively than 2D counterparts in DSS-induced colitis mice (Yang et al., J Crohns Colitis, 2022).

**5.2 Degenerative and Traumatic Diseases**

Osteoarthritis: 3D bioreactor-derived BMSC exosomes, purified via TFF/SEC, promoted cartilage regeneration and suppressed inflammation in rat OA models, attributed to higher levels of chondrogenic and anti-inflammatory miRNAs (Li et al., Bioact Mater, 2023).

Spinal Cord Injury: Exosomes from 3D-cultured hMSCs (packed-bed bioreactor, TFF+HIC) enhanced neurogenesis, reduced glial scar formation, and improved locomotor recovery in SCI rats (Hu et al., Biomaterials, 2021).

**5.3 Cancer Therapy**

Glioblastoma Targeting: 3D-cultured hMSC exosomes, engineered and harvested from scalable microcarrier-based systems, were loaded with miR-124 and selectively delivered to tumor cells, resulting in significant tumor inhibition in orthotopic mouse models (Wang et al., Theranostics, 2022).

Ovarian Cancer: 3D-hMSC exosomes produced in wave bioreactors and loaded with paclitaxel showed enhanced tumor targeting and cytotoxicity, reducing tumor burden in xenograft mice compared to free drug or 2D-exosomes (Luo et al., Acta Pharm Sin B, 2023).

**5.4 Metabolic and Cardiovascular Diseases**

Diabetic Wound Healing: Exosomes from 3D-cultured hUCMSCs (microcarrier bioreactor, TFF/SEC purified) accelerated wound closure, promoted angiogenesis, and reduced inflammation in diabetic mouse models (Zhao et al., Small, 2021).

Myocardial Infarction: hMSC exosomes derived from 3D perfusion bioreactors improved cardiac function, reduced infarct size, and promoted neovascularization in rat MI models, with enhanced efficacy versus 2D-derived exosomes (Sun et al., Bioeng Transl Med, 2022).

**5.5 Other Applications**

COVID-19: Clinical trials using hMSC exosomes prepared from 3D dynamic cultures (stirred-tank/wave bioreactors and TFF purification) have demonstrated safety and potential efficacy in reducing cytokine storm and promoting lung recovery in severe COVID-19 patients (Sengupta et al., Stem Cell Transl Med, 2021; **Table 8**).

Wound Healing and Skin Regeneration: 3D-hMSC exosomes promote collagen synthesis, angiogenesis, and epithelialization in various wound and skin defect models, outperforming 2D-derived exosomes in both efficacy and scalability (Liu et al., ACS Nano, 2022).

**5.6 Mechanistic Insights and Preparation Methods**

Across these studies, exosomes are typically produced via scalable 3D bioreactor systems (hollow-fiber, microcarrier, stirred-tank, packed-bed, and wave bioreactors), with downstream purification using TFF, SEC, anion exchange, or hydrophobic interaction chromatography to ensure clinical-grade purity. 3D culture not only increases exosome yield but also enriches regenerative, anti-inflammatory, and disease-targeting cargo (miRNAs, proteins), enhancing therapeutic outcomes.

**5.7 Clinical Translation**

Several exosome-based products in clinical development (**Table 8**) now explicitly use 3D dynamic culture and advanced purification for manufacturing. This trend reflects an industry-wide recognition that only exosomes produced under scalable, GMP-compatible 3D systems can meet the demands of clinical and commercial application.

In summary, a growing body of evidence demonstrates that exosomes generated by 3D culture systems exhibit superior therapeutic efficacy and translational potential across autoimmune, degenerative, oncologic, metabolic, and wound-healing indications.

# 6. Summary and Outlook

This review systematically highlights the advances and challenges in the field of hMSC-derived exosomes, particularly focusing on their scalable production and diverse biomedical applications. Exosomes from hMSCs retain the critical immunomodulatory and tissue repair functions of their parental cells, but with lower immunogenicity, higher safety, and improved feasibility for large-scale, standardized manufacturing—especially when produced via advanced 3D dynamic culture systems.

While the progress in 3D bioprocessing and purification technologies such as tangential flow filtration and size-exclusion chromatography has significantly improved exosome yield and purity, there remains an urgent need for further innovation and optimization. Notably, alternative and emerging isolation techniques including anion exchange chromatography and hydrophobic interaction chromatography offer additional selectivity, scalability, and potential for higher purity, yet are underexplored in the current literature and require deeper investigation and practical validation for clinical and industrial adoption.

Moreover, methods to enhance exosome yield and therapeutic potency are rapidly evolving. Recent studies have shown that modifying media composition (such as optimizing nutrients, growth factors, or using serum-free/chemically defined media), as well as genetic engineering approaches (like overexpressing exosome biogenesis regulators or therapeutic cargo), can significantly boost both exosome quantity and desired functional attributes. A more comprehensive integration of these strategies, supported by rigorous mechanistic and translational research, will be critical for the development of next-generation exosome therapeutics.

Despite substantial progress, several practical and scientific challenges must still be addressed. These include: 1) Fine-tuning high-density 3D-hMSC culture parameters and integrating external stimuli to maximize exosome yield while preserving functional quality. 2) Establishing robust, scalable, and standardized isolation and purification processes—including the systematic evaluation of alternative chromatographic and affinity methods. 3) Addressing product heterogeneity, comprehensive cargo characterization, and stringent quality control to meet regulatory and clinical requirements.

Moving forward, interdisciplinary efforts combining stem cell biology, bioengineering, process analytics, and translational medicine are essential. With continued innovation in bioprocess engineering, exosome isolation, and functional enhancement, as well as a broadened adoption of advanced and alternative purification strategies, hMSC-derived exosomes produced from optimized 3D systems are poised to become a transformative platform in precision medicine. Realizing their full clinical potential will depend on overcoming current technical barriers and establishing reliable, GMP-compliant manufacturing pipelines for safe and reproducible therapeutic products.

## Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant No. 22250410275) and also by the National Foreign Expert Program of China (Grant number: Y20240198).

## Author contributions

LWF and HYW drafted the manuscript. LWF and XKJ work together on chart organization and image optimization. AM and GMJ revised the manuscript, provides resources and funding. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was sued for the research described in this the article.

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**Figure legends**

**Figure 1 Basic information of exosomes. (A)** Extracellular vesicles or non-vesicle components in the range of 30~150 nm. **(B)** Basic components and functions of exosomes. **(C)** Biogenesis of exosomes by Figdraw.

**Figure 2 Large-scale culture of hMSCs. (A)** Example of microcarriers for hMSCs, solid microcarriers、Concave solid microcarrier、Porous microcarriers (cytoniche.com). **(B)** Stirred bioreactor and flow field simulation of stirred tank bioreactor. **(C)** Wave bioreactor and flow field simulation of wave bioreactor.

**Figure 3 Exosome isolation method: Based on exosome size and density. (A)** Ultracentrifugation. **(B)** Density gradient ultracentrifugation. **(C)** Ultrafiltration. **(D)** Size exclusion chromatography. **Based on exosome solubility.** **(E)** Polymer precipitation. Based on exosome protein specificity. **(F)** Immunomagnetic bead method. **(G)** Affinity chromatography. **(H)** Isolation methods based on exosome lipid specificity. **(I)** Microfluidics.

**Figure 4** Examples of applications of hMSCs-derived exosomes in autoimmune diseases and immune regulation, degenerative diseases, cancer, metabolic diseases, and others.