Journal of Extracellular Vesicles

LETTER TO THE EDITOR OPEN ACCESS

Quality Evaluation Considerations for Stem Cell-Derived Extracellular Vesicles-Based Therapeutic Products in China

Tao Na[1,2,3,4] | Kehua Zhang[1,2,3,4] | Libo Zhao[5] | Xuanbao Yao[1] | Chongfeng Xu[1,2,3,4] | Yuan Zhang[1] | Lan Wang[2,3] | Miao Xu[1,2,3,4] | Hui Zhang[1,2,3,4,6] | Shufang Meng[1,2,3,4]

[1]National Institutes for Food and Drug Control, Beijing, China | [2]State Key Laboratory of Drug Regulatory Science, Beijing, China | [3]Key Laboratory of the Ministry of Health for Research on Quality and Standardization of Biotech Products, Beijing, China | [4]Beijing Key Laboratory of Quality Control and Non-Clinical Research and Evaluation for Cellular and Gene Therapy Medicinal Products, Beijing, China | [5]Echo Biotech Co., Ltd., Beijing, China | [6]Center for Drug Reevaluation (National Center for ADR Monitoring), NMPA, Beijing, China

Correspondence: Hui Zhang (zhanghui@nifdc.org.cn) | Shufang Meng (mengsf@nifdc.org.cn)

Received: 13 November 2025 | **Revised:** 5 April 2026 | **Accepted:** 21 April 2026

ABSTRACT

Stem cell therapy is currently undergoing clinical research in China for conditions that are resistant to or incurable by traditional pharmaceutical interventions. Stem cell-derived extracellular vesicles (EVs) exhibit therapeutic effects similar to those of their parent stem cells, positioning them as an alternative or adjunctive approach to stem cell therapy. In recent years, given the tremendous potential for EVs in disease treatment, many researchers have focused on the development of stem cell-derived EVs and have achieved substantial progress in large-scale production and quality-related studies. However, at present, there are no specific or targeted regulatory requirements issued by authorities in China regarding the regulation of this novel therapeutic modality or the assurance of its safety and efficacy. In this paper, based on the biological properties of EVs, recent research advances, current understanding of their mechanisms of action, manufacturing processes and quality control strategies, a comprehensive framework for the quality evaluation of stem cell-derived EV-based therapeutic products is proposed. This framework is intended to serve as a reference for researchers and developers and may help to facilitate further discussion to facilitate further discussion, thereby supporting and promoting the development, regulatory oversight and establishment of quality standards and evaluation systems for stem cell-derived EVs in China.

1 | Introduction

In recent years, China has emerged as a global leader in the research and development of stem cell-based therapies, including the use of stem cell-derived extracellular vesicles (EVs) for therapeutic applications (Rahnama et al. 2024). Stem cells, particularly mesenchymal stem/stromal cells (MSCs), induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), have demonstrated tremendous potential in the treatment of various diseases, such as autoimmune conditions (Choi et al. 2025), neurodegenerative disorders (Ramalingam et al. 2025) and cardiovascular diseases (Liao et al. 2025). The therapeutic properties of stem cells are attributed not only to their intrinsic biological functions and capacity to differentiate into multiple cell types but also to their secretion of EVs (Riazifar et al. 2017).

EVs are nano- to micro-sized vesicles enclosed by a lipid bilayer that are released into the extracellular space and are incapable of replication. All types of cells, including both eukaryotic and prokaryotic cells, release EVs (Rohde et al. 2019; Kalluri LeBleu

Tao Na and Kehua Zhang contributed equally to this study.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2026 The Author(s). *Journal of Extracellular Vesicles* published by Wiley Periodicals, LLC on behalf of the International Society for Extracellular Vesicles.

2020; Hur et al. 2021; Levy et al. 2023). The lipid bilayer membrane of EVs not only protects their contents from rapid degradation but also facilitates immune evasion and enables efficient delivery of cargo to target cells. EVs carry diverse molecular cargo, including proteins, lipids, nucleic acids, metabolites and sugars, which reflect the characteristics of their parent cells. EVs mediate long-distance cell-to-cell communication by interacting with or being internalised by recipient cells, thereby releasing their cargo into these cells. Such interaction can modulate the function and metabolism of target cells, including promoting tissue repair, regulating immune responses or facilitating disease initiation and progression, such as tumour metastasis (van Niel et al. 2022).

Given their diverse biological functions and ongoing technological advancements, EVs hold significant potential for breakthroughs in diagnosis, drug delivery and disease treatment. EVs can be classified into various subtypes based on factors such as cellular origin, size, biogenesis pathways, biological functions, secretion mechanisms and applications. Based on size and biogenesis pathways, EVs are commonly classified into exosomes and ectosomes as described in the MISEV2023 guidelines (Bordanaba-Florit et al. 2021; Welsh et al. 2024). According to specific cellular processes, EVs can also be classified as apoptotic bodies, which result from programmed cell death, or migrasomes, which arise during cell migration (Welsh et al. 2024). In addition, EVs may be categorised according to their cell of origin, such as MSC-derived EVs (Kou et al. 2022), iPSC-derived EVs (Kmiotek-Wasylewska et al. 2024) or adult cardiomyocyte-derived EVs (Prieto-Vila et al. 2024). Furthermore, EV classification can be based on intracellular origin and cargo content, including mitochondrial-content-containing EVs, referred to as mitoEVs (Lou et al. 2025), or Golgi apparatus-enriched, Golgi-derived EVs (Li et al. 2025). With ongoing research, an expanding variety of EV subtypes has been identified, including large oncosomes, migrasomes, ectosomes, exomeres, supermeres and membrane particles (Cocucci and Meldolesi 2015; Kumar et al. 2024) (Figure 1). Despite substantial efforts to isolate pure exosomes, identify specific markers for exosome characterization, and elucidate their biological functions, exosomes cannot yet be unequivocally distinguished from other EVs using currently available characterization methods. Therefore, it may be appropriate to use the broader term 'extracellular vesicles' or 'small extracellular vesicles (sEVs)' when their size distribution is strictly controlled within appropriate range (e.g., less than 200 nm) for therapeutic products unless the subcellular origin and exact proportion of exosomes can be precisely demonstrated (Zhou et al. 2025).

Currently, the development of stem cell-derived EVs-based therapeutic applications has gained significant attention. Globally, thousands of researchers are engaged in the development of therapeutic EV products, and dozens of EV-based products are currently in various stages of clinical trials, several of which are expected to enter the market in the near future (Mizenko et al. 2024). Numerous preclinical studies have demonstrated that EVs hold substantial promise for disease treatment. EVs are being explored as carriers for the delivery of small molecule drugs, proteins, genes and other bioactive substances, enabling targeted delivery to specific cells for therapeutic purposes (Huang et al. 2025). Owing to their lipid bilayer structure, EVs possess distinct advantages over other nano carriers, including the ability to penetrate biological barriers, such as the blood-brain barrier (Mehdizadeh et al. 2025). Compared with synthetic nanoparticles, such as lipid nanoparticles (Herrmann et al. 2021), EVs demonstrate superior capabilities in targeting specific cells, tissues and organs, as well as enhanced efficiency in crossing both extracellular and intracellular membranes. This unique property enables EVs to mediate the delivery of functional molecules, including chemicals, nucleic acids, proteins and lipids—from one cell to another, even across long distances within the body (Le Saux et al. 2021).

While significant progress and promising results have been achieved in EV research, the application of EVs as therapeutic products remains at an early stage compared with stem cell-based therapies. Challenges persist, particularly in the quality evaluation of therapeutic EV products (Rahnama et al. 2024). As research advances, various countries have begun developing frameworks and guidelines to address the challenges associated with EVs, particularly with respect to production, quality control, characterization and regulatory approval for clinical use. Although no global standard currently exists, several countries have made notable progress in establishing quality evaluation frameworks, setting a precedent for other nations to develop similar standards. According to US regulations of the Public Health Service (PHS) Act Section 351 (HCT/P), EV products are regulated as human cells, tissues and cell- and tissue-based products. This classification includes stem cell-derived EVs and provides recommendations on manufacturing controls, quality assessment, characterization, safety and mechanisms of action (Wang, et al. 2024). To evaluate the effectiveness and safety of EVs in humans, the US Food and Drug Administration (FDA) emphasises the need for additional clinical trials (https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/public-safety-notification-exosome-products). Furthermore, due to the lack of standardised isolation and analytical procedures, EV-based therapies have not yet been approved by the FDA (Yousefian et al. 2024). On 11 July 2025, the biotechnology firm Capricor Therapeutics announced that its Biologics License Application (BLA) for Deramiocel—a cellular therapy based on exosome-mediated mechanisms developed for the treatment of cardiomyopathy associated with Duchenne muscular dystrophy (DMD)—was not approved by the FDA because it failed to meet the statutory requirement for substantial evidence of effectiveness. This case further underscores the FDA's continued emphasis on the need for robust evidence to substantiate the safety, efficacy and mechanism of action of EV-based therapies (https://www.biospace.com/fda/capricor-plunges-on-fda-rejection-of-dmd-cell-therapy). Although the European Medicines Agency (EMA) does not have specific guidelines exclusively for EVs, it classifies them under the broader category of biologics or advanced therapy medicinal products (ATMPs), depending on whether the EVs contain functional translated RNA (EMA Scientific recommendations on classification of ATMP: https://www.ema.europa.eu/en/human-regulatory-overview/marketing-authorisation/advanced-therapies-marketing-authorisation/scientific-recommendations-classification-advanced-therapy-medicinal-products). In Japan, EVs that do not contain living cellular components are not considered regenerative medical products or medical devices;

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

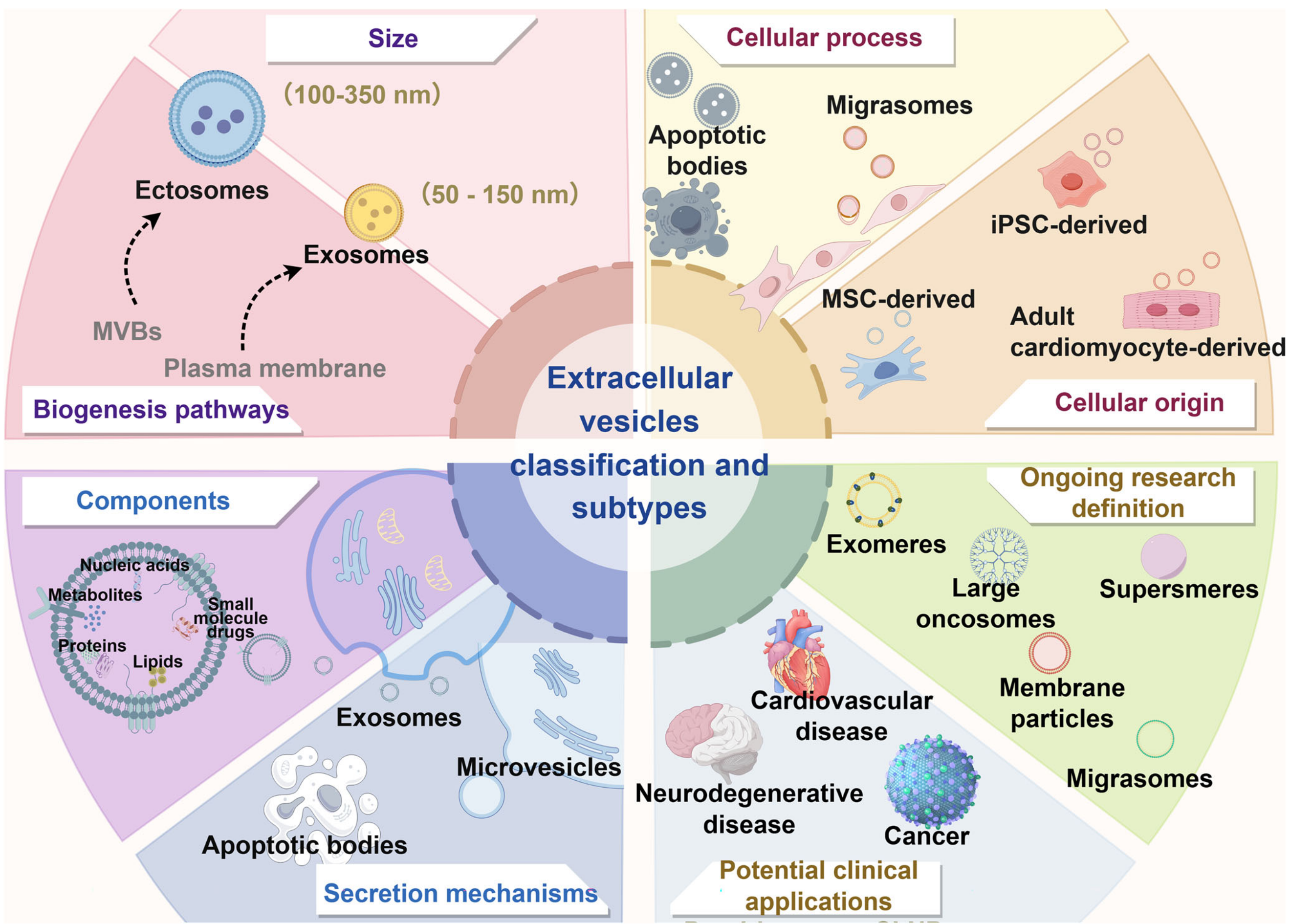

FIGURE 1 | Extracellular vesicles (EVs) can be classified into several subtypes based on factors, such as size and biogenesis, cellular processes and cell of origin.

instead, they are classified as biologics (Tsuchiya et al. 2022; Wang, et al. 2024).

Driven by a rapidly advancing biomedical industry, substantial investments in stem cell and regenerative medicine research, and a growing demand for advanced medical therapies, China has made significant progress in both basic and clinical research on stem cell-derived EVs (Wang et al. 2023; Zhang et al. 2023). According to data from ClinicalTrials.gov, China is now the second-largest country in terms of registered EV clinical trials, surpassed only by the United States. Among the 161 registered studies, 42 of them focus on therapeutic EVs, while the remaining studies investigate the use of EVs as biomarkers for disease diagnosis, treatment prognosis or clinical status assessment. With continuous advancements in EV research, substantial efforts have been made to accelerate the development of therapeutic EVs. For example, from 2016 to 2022, only 11 therapeutic EV studies were registered on ClinicalTrials.gov; however, in nearly 3 years since 2023, this number has increased to approximately three times the previous total (Figure 2A). Geographically, therapeutic EV research is primarily concentrated in Shanghai, Guangdong Province and Beijing (Figure 2B). Among the currently registered therapeutic EV studies, over 95% involve natural stem cell-derived EVs, with MSCs accounting for the largest proportion, representing approximately 60% of registered therapeutic EVs (Figure 2C). Among these MSCs-derived EVs, 12% are adipose-derived EVs, 44% are umbilical cord-derived EVs and the remaining 44% are of unspecified origin due to incomplete registration information, precluding further source analysis (Figure 2D). Beyond therapeutic EVs, other EV-related applications and research areas have also progressed relatively rapidly in China. For instance, in 2024, EV-based cosmetic products were not approved for registration because they did not comply with existing regulations and technical specifications. In addition, the National Institutes for Food and Drug Control (NIFDC) issued a notice explicitly recommending that EVs should not be regulated as medical devices (https://www.nifdc.org.cn/nifdc/bshff/ylqxbzhgl/qxxxgk/fljd/20241031105830130l565.html).

To address emerging products such as EVs, the Centre for Drug Evaluation (CDE) of the China National Medical Products Administration (NMPA) issued the 'Scope, Classification, and Interpretation of Advanced Therapeutic Medicinal Products (ATMPs) (Draft for Consultation)' on 10 June 2025 (https://www.cde.org.cn/main/news/viewInfoCommon/0d19d9228c90f124053e92cda08331e0). This document clarified that EVs fall within the regulatory scope of ATMPs. Moreover, China's regulatory landscape has continued to evolve to support innovation in this field, though challenges remain in developing specific frameworks for the quality control and clinical application of these products (Lu et al. 2024). To promote the research and regulatory development related to EVs in China

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

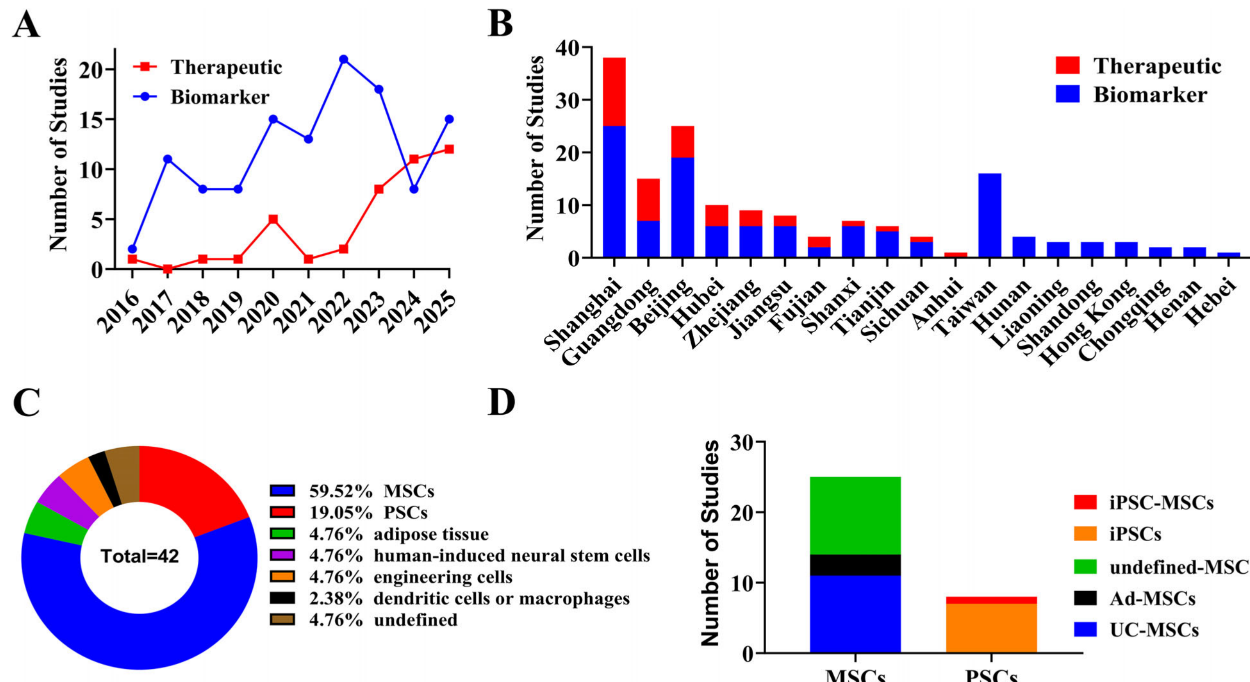

FIGURE 2 | Statistic overview of EV clinical studies registered on ClinicalTrials.gov by institutions in China. (A) Annual number of EVs studies registered between 2016 and 2025 (Therapeutic refers to EVs registered for therapeutic purposes; Biomarker refers to studies using EVs as biomarkers for disease diagnosis, treatment prognosis or clinical status assessment.). (B) Geographic distribution of EV clinical studies registered in China, with therapeutic EV ranked from highest to the lowest by number. (C) Distribution of cell sources among registered therapeutic EVs. (D) Distribution of stem cell-derived EVs used for therapeutic purposes. MSCs, mesenchymal stem/stromal cells; PSCs, pluripotent stem cells; undefined, EVs with incomplete registration information; iPSCs, induced pluripotent stem cells; Ad-MSCs, adipose-derived MSCs; UC-MSCs, umbilical cord-derived MSCs. (Data are current through September 2025.).

and to establish appropriate quality evaluation systems, this article focuses on the most extensively studied stem cell-derived EVs (Figure 3). Quality requirements for different types of stem cell-derived EVs primarily differ across multiple aspects while ensuring the safety and efficacy of the corresponding EV products. Therefore, quality control strategies can be tailored to the specific characteristics of each type of stem cell-derived EVs, with corresponding requirements and methods developed accordingly.

Based on the biological properties of EVs, current research progress, and the growing understanding of their mechanisms of action, manufacturing processes and quality control strategies (Xu et al. 2025), detailed considerations for the quality evaluation strategies of stem cell-derived EV products have been proposed for reference. These strategies do not represent formal regulatory guidelines but are intended solely as a reference for researchers and developers working on EV-based products. The proposed strategies may provide a reference framework for systematic quality assessment and may also contribute to ongoing discussions on regulatory alignment and the clinical development of EV products in China.

2 | Considerations for Quality Evaluation of Stem Cell-Derived EV Products

The proposed considerations for the quality evaluation of stem cell–derived EV products are provided as a reference to encourage more systematic and comprehensive investigations of EV properties. Such efforts may facilitate the identification of additional relevant critical quality attributes (CQAs) and ultimately improve the overall quality and consistency of EV-based therapeutics. According to the ICH Q8–Q10 framework (International Council for Harmonizations of Technical Requirements for Pharmaceuticals for Human Use, available from: https://www.ich.org/page/quality-guidelines), CQAs for EV products are established during development based on the quality target product profile and risk assessment, and are subject to continual verification and refinement throughout the product lifecycle. In alignment with ICH Q10 and Q12, post-approval quality research and lifecycle management enable dynamic adjustments of CQAs and quality specifications as new knowledge and clinical evidence emerge. As deeper insights into structure–function–clinical correlations are gained, CQAs may be redefined or reprioritised, thereby driving corresponding refinements in quality specifications and control strategies. This iterative process underpins lifecycle quality management and ensures sustained product consistency, safety and therapeutic efficacy over time.

2.1 | Stem Cell Bank System

Quality control of raw materials is a fundamental component of the production of stem cell-derived EVs. In this context, stem cells should be regarded as the primary raw material for EV production, as the content and biological properties of EVs may reflect the specific characteristics and functions of their parent

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

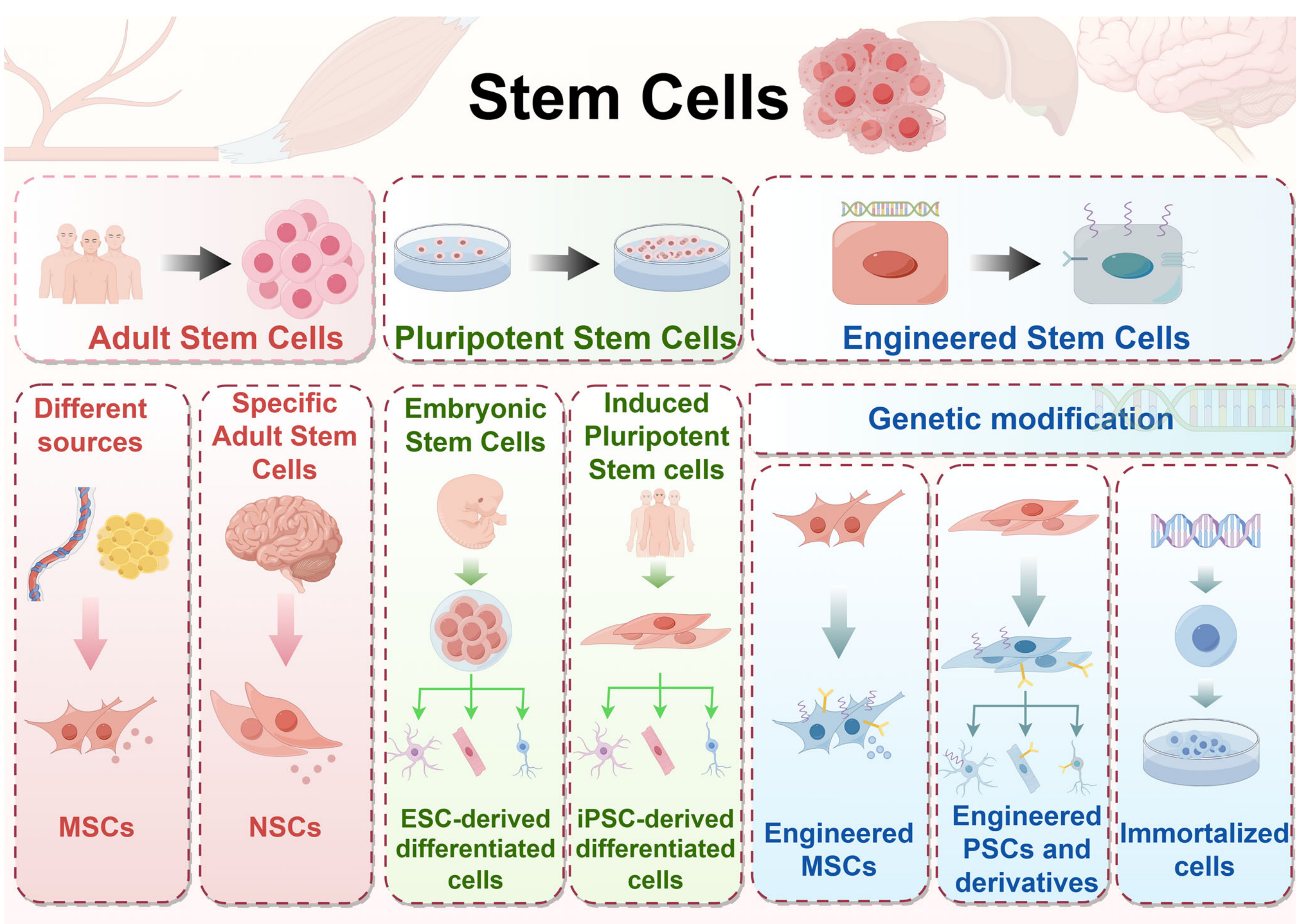

FIGURE 3 | Classification of stem cells used for therapeutic EVs production. In this paper, the term 'stem cell' includes adult stem cells (also referred to as somatic stem cells or tissue stem cells), such as MSCs, adult neural stem cells; pluripotent stem cells (PSCs), including ESCs and iPSCs, and various types of PSC-derived cells. All stem cells involving genetic modification are collectively categorised as genetically modified stem cells.

stem cells. Establishing a stem cell banking system is therefore essential to ensure consistent quality across different batches of EV production. It is generally advisable that developers evaluate the suitability of stem cells for EV product development prior to the establishment of a cell bank. For example, when allogeneic MSCs are used, the primary cells suitable for MSC-based cellular therapies may differ from those optimal for EV product development. For MSCs intended for EV production, a comprehensive evaluation of key parameters—such as the average level of EV secretion and the stability of EV production across passages—is considered necessary. Before cell bank establishment, stem cells are thoroughly characterised and validated through rigorous testing to ensure the safety and efficacy of the resulting EV products for their intended therapeutic application. In addition, stem cells subjected to genetic editing, reprogramming or extensive in vitro passaging may undergo morphological, phenotypic or genetic alterations (Yang et al. 2018; Turchiano et al. 2021; Vaz et al. 2021). These changes may affect the quality and yield of stem cell-derived EVs. During the establishment of a cell bank, genetically modified stem cells therefore undergo comprehensive quality evaluations, including genetic stability assessments, to confirm the absence of abnormalities. For PSC-differentiated cells, both the quality of the PSCs and that of the corresponding differentiated cell banks may be evaluated. However, the limited proliferative capacity of certain PSC terminally differentiated cells may render them unsuitable for cell bank establishment. In such cases, the quality of terminally differentiated cells used directly for EV production may be directly assessed.

In China, stem cell banks used for therapeutic EV production are subject to regulatory requirements, such as the Chinese Pharmacopoeia and the 'Technical Guideline for Pharmaceutical Research and Evaluation of Human Stem Cell Products (for Trial Implementation)' (https://www.cde.org.cn/main/news/viewInfoCommon/1dfacaa7804aca84d648edb83b10c40b). Stem cell banks are required to undergo testing for cell identity, sterility, mycoplasma, exogenous and endogenous contaminants, cell viability, tumourigenicity, cytogenetics stability and cell-specific biological functions, among other parameters (Table 1). Only stem cell banks that meet these requirements are eligible to serve as a cell source for therapeutic EV production. The quality control contents listed in Table 2 for each type of cell bank are provided for reference only. The specific tests to be conducted are determined based on factors such as the cell manufacturing process, cell type and the quantity of cells available.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 | Considerations for quality control of stem cell bank for EVs production.

Quality control contents of Stem cell bank for EVs production		Recommendations
1. Identity test	Morphology	It is generally considered to be consistent with the characteristics of specific cell type.
	Source identification	Includes species, individual or tissue origin, as appropriate for the specific cell type.
	Cell markers	Semi-quantitative or quantitative testing may be performed. The selected markers are appropriately expressed and meet the acceptance criteria. In addition, negative markers for potential unrelated cell types are included in the testing panel.
	Inter-species cell contamination testing	Should be negative.
	Genetic modified	Testing is used to detect the insertion site(s), copy number, and expression of the target gene.
2. Test for sterility	Bacteria and fungi	Using methods in accordance with the ChP.
3. Test for mycoplasmas	/	
4. Test for adventitious and endogenous viruses	In vitro assay	Using methods in accordance with the ChP.
	In vivo assay	
	Retroviruses	
	Species-specific viruses	Testing should be based on species-related viruses that may be introduced during cell isolation, acquisition, culture, expansion, passage and cryopreservation. Methods in accordance with the ChP should be used.
	Other selected viruses	Viruses specifically used for cell line development such as reprogramming or gene editing should be tested. Viruses specifically used during cell line development, such as those involved in reprogramming or gene editing, should be tested.
5. Biological function evaluation	General biological function	Biological functions that are expected to be possessed by the specific cell type.
	Specific biological function	To detect changes in the specific biological functions of stem cells resulting from genetic modification.
6. Test for cell activity	Cell viability, cell cycle, etc.	Cell activity is required to meet the requirements for EV production.
7. Test for tumourigenicity	In vitro assay	Testing can be conducted, particularly for genetically modified cells.
	In vivo assay	
8. Test for oncogenicity	In vitro assay	
	In vivo assay	
9. Genomic stability[a]	Multiple methods	Tests are conducted at multiple levels using karyotyping, whole-exome sequencing (WES) and transcriptomic analyses. The tested cell passage number is expected to be at least equivalent to the maximum passage number expected to be reached at the end of the EV production process.
10. Residue testing	Gene editing-related residues	Materials used in the gene editing process, including plasmids and small molecules, are assessed.
	Residual cells	For pluripotent stem cell-derived differentiated cells, testing is conducted to detect residual undifferentiated cells, partially differentiated cells, or abnormally differentiated cell types.
11. Test for gene editing (for genetically modified stem cells)	Editing efficiency	In addition to assessing editing efficiency and off-target effects, it is also important to consider whether gene modification or off-target effects alter cellular functions and the components of the secreted EVs.
	Off-target effects	

Abbreviation: ChP, Chinese pharmacopoeia.
[a]Genetic modified stem cells and PSCs are tested.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 | Recommendations for quality control of stem cell banks used for EV production.

Quality control contents	PCB	MCB	WCB
1. Identity test	+	+	+
2. Test for sterility	+	+	+
3. Test for mycoplasmas	+	+	+
4. Test for adventitious and endogenous viruses	—	+	—
5. Biological function evaluation	—	+	—
6. Test for cell activity	—	+	+
7. Test for tumourigenicity	—	—	+
8. Test for oncogenicity	—	—	+
9. Genomic stability	+	+	+
10. Residue Testing	+	—	—
11. Test for gene editing (for genetically modified stem cells)	+	—	—

Note: +: Required to test; –: Not mandatory, select tests based on the actual situation.
Abbreviations: MCB, master cell bank; PCB, primary cell bank; WCB, working cell bank.

2.2 | Stem Cell-Derived EVs

EVs are a diverse group of nanoparticles that vary in size, composition and function. Multiple factors can affect the quality and yield of EVs, including technical aspects such as isolation and quantification procedures, as well as biological factors such as cell type, culture medium composition and culture conditions (Gudbergsson et al. 2016). Therefore, it is essential to perform a comprehensive quality evaluation of EVs to assess their safety and efficacy from multiple perspectives, taking into account different quality attributes, multiple aspects within each attribute, as well as product consistency and stability.

At a minimum, quality evaluation typically includes characterization, physicochemical properties, exogenous and endogenous contamination, strength, purity, biological functions and safety. Together, these quality attributes provide a comprehensive profile of isolated EV products, offering insights into their heterogeneity, origin, purity, potential contamination and desired biological functions.

2.2.1 | Identity

The identity of stem cell-derived EVs is considered a critical component of their quality evaluation framework. All relevant characteristics of EVs are thoroughly considered to ensure effective quality control for subsequent therapeutic purposes, including safety, efficacy and reproducibility in clinical applications.

EVs contain fragments of cellular genomic DNA as well as mitochondrial DNA (Pegtel and Gould 2019). Identification of the individual origin of EVs ensures the correct source cell and preservation of its unique biological properties for therapeutic applications. EVs derived from different cell sources exhibit different characteristics and functions, and a better understanding of these differences may provide new opportunities for precision therapy (Jiao et al. 2024). Takakura et al. suggested that pooling cells from multiple donors and establishing a cell bank may be preferable, as MSCs and other stem cells can vary depending on donor source, and their properties may also differ between batches (Takakura et al. 2024). Consistently, other studies reported that pooling MSCs from different donors reduced heterogeneity and yielded MSCs with more consistent functional profiles (Kannan et al. 2024). Similarly, pooling individual EV samples was shown to reduce biological variation, although this approach may mask the biological significance of individual EVs populations (Raj et al. 2012). However, more recent evidence indicated that pooling MSCs from multiple donors does not reduce inter-donor variability or heterogeneity; rather, it obscures these differences, while only reducing the variability of the obtained data (Kukaj et al. 2025). In addition, pooling stem cell-derived EVs from multiple donors may introduce potential risks, including immunogenicity and other safety concerns. Therefore, to ensure the safety, traceability and functional stability of EV products, pooling EVs derived from different cell sources may not be advisable. Instead, greater emphasis can be placed on ensuring functional consistency of EV across production batches derived from well-characterised and traceable cell sources. To confirm the individual cellular origin of EVs and exclude contamination from other donors or human cells, short tandem repeats (STR) profiling can be employed (Tanudisastro et al. 2024).

Currently, in most reported studies, EVs characterization based on protein composition generally follows the MISEV2023 recommendations (Welsh et al. 2024). However, these protein markers are largely shared across different cell types and therefore cannot provide specific information regarding the individual donor, cell type, tissue origin or genetic modification status of EVs. Such information is particularly crucial for stem cell-derived EV-based therapeutic products, as it is closely associated with product efficacy and safety. Accordingly, phenotypic characterization of EVs can be divided into two categories: general markers and specific markers.

For general EV markers, the five categories of protein components suggested in by MISEV2023 remain appropriate. Based on

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

current evidence, the use of confirmed non-EV components as negative markers (Welsh et al. 2024) may also be considered to assess purity and control potential impurities. These negative markers can be classified into three groups: (i) major components co-isolated with EVs but not originating from EV (Huang et al. 2021); (ii) proteins associated with intracellular structures other than the plasma membranes and endosomes (Iovine et al. 2021); and (iii) secreted proteins that are non-specifically recovered during EV isolation (Badosa et al. 2023). Importantly, there are currently no universal markers that are present in all EV populations or across all intracellular EV biosynthetic pathways (Fordjour et al. 2022). Proteomic studies of EVs have demonstrated that commonly used tetraspanins, including CD9, CD63 and CD81, are not uniformly expressed in all EVs, and that EVs derived from different cell types exhibit distinct proteomic features (Kugeratski et al. 2021). Therefore, tetraspanins such as CD9, CD63 and CD81 are not used as sole indicators of EV purity. Instead, the expression frequency of individual markers or defined co-expressed patterns may be considered indicators of process stability for the quality control of EVs produced from the same donor source or manufacturing process. With rigorous validation, the expression levels of these general EV markers, either individually or in combination, may serve as efficacy-related quality attributes, provided that they can be correlated with the functional activity of EV products.

It may be appropriate to establish targeted positive and negative markers based on specific source cell type and production process for quality control. In early studies of MSC-derived EVs, surface markers such as CD73 and CD105 were proposed for EV characterization (Rohde et al. 2019). In 2024, Nguyen et al. 2024 identified CD73, CD105 and CD44 as robust positive specific markers for minimally identifying MSC-derived EVs, while CD11b, CD14, CD19, CD45 and CD79 were proposed as reliable negative markers. These findings were based on a novel multiplex bead-based EV flow cytometry assay panel involving 11 different MSC-EV products from five laboratories (Nguyen et al. 2024). In 2024 Chen et al. confirmed that PODXL and SSEA4 could serve as specific markers for PSC-derived EVs, with a positivity rate exceeding 70%, as demonstrated through proteomic analysis, immunoblotting screening, and validated by nano-flow cytometry (Chen et al. 2024). Subsequently, in 2025, the same research team identified CD13, CD29 and CD90 with a positive rate exceeding 60% in MSC-sEV as putative markers (Luo et al. 2025). Negative markers play a critical role in excluding non-target vesicles or non-vesicular components introduced during procedures such as EV isolation and purification, and mitigating potential residual risks, including viral particles that may persist following genetic modification of source cells via viral transduction.

Currently, there is no unified standard for the antigen positivity rates of marker molecules on stem cell-derived EVs. Positivity rates are influenced by multiple factors, including the source of the cells, cell and EV manufacturing processes, EV isolation methods, detection techniques, analytical instruments, and the specificity and affinity of the antibodies used. In the absence of mature and validated reference methodologies, developers can establish internal detection standards based on data derived from multiple production batches, in accordance with the specific manufacturing processes of their products. Furthermore, these standards are correlated with other relevant quality attributes, such as efficacy for specific indications, membrane integrity and impurity profiles. This integrated approach would enable developers to define appropriate thresholds for marker positivity rates, establish product-specific quality control criteria, and determine lot release standards, thereby ensuring the efficacy and safety of EV-based therapeutic products.

For genetically modified stem cell-derived EVs, it is essential to evaluate whether the EVs contain the genetic modification targets, particularly when these targets are closely associated with EV properties or biological functions. In such cases, the proportion of EVs positive for the genetic modification needs to be quantitatively assessed. For PSC-derived EVs, those originating from spontaneously differentiated PSCs are also taken into consideration. In addition, the presence of EVs secreted by undifferentiated cells or cells undergoing unintended differentiation is characterised and evaluated in the final EV product. If direct evaluation of these EVs subpopulations is not feasible due to their low abundance or insufficient characterization, the proportion of the corresponding cell populations among all EV-producing cells can, at a minimum, be assessed and predefined during the EV harvesting process.

2.2.2 | Physicochemical Properties

The size of EVs typically ranges from 30 to 150 nm, as reported in most current therapeutic studies. This size range falls below the diffraction limit of conventional optical microscopes (Noble et al. 2020), rendering optical microscopes unsuitable for obtaining clear images of EVs. Consequently, EV morphology is primarily observed using electron microscopy-based techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cryo-electron microscopy (cryo-EM) (Shao et al. 2018). Vacuum conditions and the fixation and dehydration steps involved in sample preparation for electron microscopy analysis often result in a characteristic cup-shaped appearance of EVs, which may not accurately reflect their native morphology may lead to misinterpretations (Wu et al. 2015; Jeppesen et al. 2019). Therefore, morphological assessment of EVs emphasises the verification of the presence and integrity of the lipid bilayer, rather than focusing solely on vesicle shape. Indeed, evaluation of lipid bilayer integrity represents a critical parameter EV morphology characterization.

The size distribution of EVs is a critical quality attribute in EV characterization. Multiple factors, including isolation methods, detection instruments, sample concentration, the proportion of vesicles with different sizes and the sampling volume used for analysis, can influence the measurement of EV size distribution (Woo et al. 2016; Vestad et al. 2017; Comfort et al. 2021; Williams et al. 2023). Within the size range of 30–150 nm, the surface area and volume of the largest vesicles theoretically differ from those of the smallest vesicles by up to 25- and 125-fold, respectively. Therefore, accurate determination of EV diameter distribution and the corresponding particle concentration is essential to minimise heterogeneity arising from measurement variability and to ensure batch-to-batch consistency of EV products.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

To ensure measurement accuracy, it may be appropriate to validate the instrument used for size determination prior to analysis. Validation may include the use of calibrated nanoscale reference standards to assess the working size range, accuracy, precision and limit of detection (LOD) of the instrument, such as Nanoparticle Tracking Analysis (NTA), Tunable Resistive Pulse Sensing (TRPS) or nano-flow cytometry. In addition, the instrument's resolution can be validated when analysing EV samples with continuous size distributions. Mixtures of size-standards with defined but varying proportions are used to better mimic the continuous diameter distribution characteristic of EV samples. Unlike EVs, which are enclosed by a lipid bilayer, commercially available nanoscale standards are typically composed of materials with different physicochemical properties, such as silicon dioxide or polystyrene. Therefore, beyond instrument validation, reference EV preparations can be established with physicochemical properties comparable to those of the test samples. This approach may help minimise measurement artefacts arising from difference in refractive index and other intrinsic material properties between artificial size standards and EV samples (Chandler et al. 2011; Koritzinsky et al. 2017). Furthermore, the application of orthogonal measurement approaches using different analytical platforms is encouraged, as this strategy can reduce biases introduced by the inherent limitations and underlying principles of individual measurement technologies (Arab et al. 2021).

Genomic analysis has revealed that EV subpopulations within different size range, such as large EVs (90–120 nm) and small EVs (60–80 nm), possess unique biological and physical characteristics, including differences in N-glycosylation patterns, proteins composition, lipids profiles, nuclei acids content. Moreover, these EV subpopulations exhibit different distribution patterns in various organs, suggesting distinct biological functions (Zhang et al. 2018). Consistently, studies have also shown significant differences among EV subpopulations within defined size ranges in cardiac repair responses. Cardiac progenitor cell-derived middle-sized and smallest-sized sEV subpopulations exhibited the highest pro-angiogenic and anti-fibrotic activities, while the largest-sized subpopulation showed no effect on any of the functional assays (van de Wakker et al. 2024). Therefore, when experimental evidence indicates that EV size distribution is correlated with their specific biological functions, size range–defined EV subpopulations are considered as distinct quality attributes, and corresponding specifications may be established for these subpopulations. For size distribution analysis, vesicles outside the designated size range are also evaluated, as their relative proportions can reflect EV purity from a size-based perspective. Previous studies suggest that particles smaller than 50 nm in diameter are predominantly non-vesicular extracellular particles (NVEPs), including protein complexes, lipoproteins or viral particles (Zhang et al. 2018). Accordingly, although size distribution represents an important quality attribute for EV characterization, it is not sufficient as the sole criterion for demonstrating EV purity.

Owing to their high surface-to-volume ratio, EVs are more strongly affected by electrostatic stabilization mechanisms than larger particles. Zeta potential is therefore an important parameter reflecting the colloidal stability of EV dispersion. A high absolute zeta potential value indicates strong electrostatic repulsion between EVs, which helps prevent aggregation and maintain colloidal stability or adhesion to other available surfaces (Midekessa et al. 2020). Previous studies have shown that the zeta potential of EV suspensions decreases with increasing buffer ionic strength. In contrast, the presence of surfactants (such as Tween-20) or acidic pH conditions can increase the magnitude of the negative zeta potential. Furthermore, cations such as Na^{+}, K^{+} and Ca^{2+} reduce the negative zeta potential of EVs, with this effect positively correlated with cation valence (Midekessa et al. 2020). Notably, a recent study reported significant associations between the expression levels of miRNA-30 and miRNA-155 and the zeta potential of EVs (Mendivil-Alvarado et al. 2023). Collectively, these findings indicate that zeta potential not only affects the physicochemical stability of EVs but may also represent a critical quality attribute associated with EV biological function.

2.2.3 | Strength

The strength of EV products is typically expressed as the amount of a specific active component per defined number of EV particles, reflecting the quantification of a particular functional attribute of the EV product. This parameter may indicate the biological activity or therapeutic potential of stem cell-derived EVs. EV concentration represents a fundamental quality attribute related to EV strength, as EVs exert their biological functions through intercellular communication by delivering diverse bioactive molecules, including proteins, lipids and nucleic acids (Tian et al. 2022; Li et al. 2024; Wang, et al. 2025; Wang, et al. 2023). Given the complexity and heterogeneity of EV cargo, their biological activities are often mediated through multiple mechanisms. Therefore, when specific bioactive molecules are rigorously validated and demonstrated to be directly associated with the intended biological function or therapeutic indications, the quantified level of such components may be considered a potency-related parameter of EV products. Based on comprehensive functional and analytical studies, quality standards for EV strength can be established and applied as one of the key release criteria for therapeutic stem cell-derived EV products.

Statistical data from preclinical and clinical studies suggest that EV dosing is primarily determined based on particle concentration, EV protein content or the total amount of specific active components, such as marker protein or nucleic acids (e.g., miRNA) (Gupta et al. 2021). Webber and Clayton further demonstrated that a particle-to-protein ratio greater than 3×10^{10} particles per microgram protein has been regarded as indicative of high vesicular purity, whereas a reduced ratio generally reflects the presence of contaminating protein (Webber and Clayton 2013). Accurate determination of EV concentration is essential and is considered a fundamental requirement for appropriate dose definition, assessment of long-term storage stability, and calculation of intended dilutions prior to administration. In addition, EV concentration is incorporated as a critical quality attribute for evaluating the potency and safety of EV-based products. Despite its apparent conceptual simplicity and broad recognition, precise quantification of EV concentration remains technically challenging and therefore requires careful methodological consideration. Accordingly, validated analytical platforms may be used for EV concentration measurement,

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

including NTA (Comfort et al. 2021), TRPS (Vogel et al. 2016) or nano-flow cytometry (Tian et al. 2020). However, EV concentration alone is insufficient for interpretation and can be evaluated in conjunction with size distribution, as this combined assessment more accurately reflects EV biological strength and functional relevance (van der Pol et al. 2014). When evidence demonstrates that a specific size range is closely associated with EV potency, it may be appropriate to restrict EV quantification to particles within the defined size range, rather than reporting total particle counts alone. Based on these considerations, instrument validation requirements for EV concentration measurements prior to analytical assays are aligned with those established for size distribution analysis, to ensure data robustness, reproducibility and comparability across studies.

To accurately determine EV concentration, a nanoparticle reference standard with a known concentration is required (Welsh et al. 2020). Unlike size standards, there is a shortage of nanoparticle references with certified particle number concentration values (Farkas et al. 2025). The most widely used method for determining the particle number concentration of a nanoparticle reference standard with uniform material is to calculate it by dividing the total mass by the particle volume and density (Shang and Gao 2014). To accurately determine EV concentration, the use of nanoparticle reference standards with well-defined and traceable particle number concentrations may be appropriate (Welsh et al. 2020). However, in contrast to size reference materials, there remains a significant lack of nanoparticle standards with certified particle number concentration values, which limits the robust evaluation of measurement accuracy (Farkas et al. 2025). For nanoparticle reference materials composed of homogeneous and uniform particles, the most applied approach for estimating particle number concentration is to calculate it by dividing the total particle mass by the volume and density of individual particles (Shang and Gao 2014). Despite the validation of detection instruments, EV concentration determined using calibrated analytical platforms is not assumed to represent true particle concentration without further confirmation. Therefore, EV concentration measurements may be complemented by corresponding biological activity or functional studies, which can provide an objective assessment of EV strength under varying conditions. Such studies are particularly important when evaluating factors that may influence particle concentration measurements, including long-term storage, formulation strategies and the incorporation of excipients, all of which have the potential to affect the accuracy and reliability of particle-based assays.

The total amount of protein, nucleic acids and lipids may not always directly serve as reliable strength markers for EVs. When considering the quality attributes to strength, multiple factors are taken into account. EVs typically contain cell membrane proteins, signalling molecules, enzymes and other bioactive components. These proteins determine the functional characteristics of EVs, such as cell recognition and signal transduction (Sanghvi et al. 2025). The activity and stability of proteins within EVs are crucial for their biological functions (Ahmadian et al. 2024). Therefore, once a target protein has been identified and validated, it is necessary to quantify the protein as a marker of EV strength (Cvjetkovic et al. 2016); this principle also applies to other functionally relevant target components as well. Given the complexity of EV protein composition, which is strongly influenced by factors such as donor variability, cell types and culture conditions, functional studies are required in addition to quantifying the target protein at the intended therapeutic dose (Zaborowski et al. 2015; Vagner et al. 2019). This combined approach ensures that protein abundance and functionality meet the requirements of the intended application. Nucleic acids, particularly RNA species (e.g., miRNA, mRNA, etc.), are highly susceptible to degradation. Therefore, in addition to quantifying the target RNA, integrity assessments are also performed (Schroeder et al. 2006; Miceli et al. 2024). Similar to protein-based markers, it is essential not only to measure the amount of the target nucleic acid but also to verify that the RNA content within EVs meets the functional requirements of the intended application (Lee et al. 2020; Sivanantham et al. 2022).

Lipids are crucial components of the EV membrane and play key roles in determining EV stability, delivery capacity, targeting ability and other functional properties (Skotland et al. 2020, 2023). The lipid composition of EVs is generally associated with the membrane characteristics of their source cells. Different lipid classes (such as phospholipids, sphingolipids) significantly influence membrane fluidity, stability and overall EV functionality (Lai and Lim 2019; Fyfe et al. 2023; Ghadami and Dellinger 2023). Therefore, when lipid components are considered as strength-characterising attributes, their effects on EV targeting ability and biocompatibility are systematically evaluated to determine whether they meet predefined performance criteria.

Degradation processes occurring during storage following EV preparation can adversely affect the quality of strength characterization components (Ahmadian et al. 2024). Consequently, the stability and functional integrity of these components are thoroughly investigated, and appropriate release specifications are established to ensure that EV products remain safe and effective throughout long-term storage (Jeyaram and Jay 2017; Görgens et al. 2022; Yang et al. 2024).

2.2.4 | Adventitious Contamination

The source cells used to produce stem cell-derived EVs are strictly managed in accordance with established cell bank requirements. As a result, the risk of contamination originating directly from source cells is generally low. However, during downstream processes, including EV isolation, purification and formulation, EVs may be exposed to various types of contaminants that can adversely affect their safety and efficacy (Raab-Traub and Dittmer 2017; Pham et al. 2021; McConnell et al. 2022; Wang, et al. 2024). Such contaminants not only compromise EV quality but may also lead to serious adverse effects. For example, pathogenic viruses have been reported transmissible via EVs (Giannessi et al. 2020).

Therefore, particular attention is focused on adventitious contaminants that may be introduced during EV isolation, purification and formulation as well as reagents, equipment or contact materials used in the manufacturing process (Stolk and Seifert 2015; McConnell et al. 2022; Wang, et al. 2024). Among these,

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

the most common microbial contaminants include bacteria, fungi, mycoplasma and endotoxins. Mycoplasma contamination may alter the immunological activity of EVs (Quah O'Neill 2007), while endotoxin can significantly interfere with—or even obscure—the true biological effects of EVs (Babula et al. 2023). These contaminants are therefore routinely tested in accordance with the requirements for therapeutic products and relevant specifications. In addition, the potential risk of viral contamination related to the source species should be carefully evaluated (Pham et al. 2021) when human- or animal-derived reagents are used during EV production. Because viruses are often similar in size to EVs, they are particularly difficult to detect and may be overlooked due to unknown origins or limitations of conventional detection methods (Nolte-'t Hoen et al. 2016; Raab-Traub and Dittmer 2017; Yang et al. 2021; Moulin et al. 2023). Accordingly, the use of in vitro non-specific virus detection assays may be considered, and the application of next-generation sequencing (NGS) technologies is being explored to enable comprehensive detection of potential exogenous viral contaminants (Tesovnik et al. 2021; Chow and Morphew 2023).

Beyond microbial contamination, consideration is also given to foreign substances derived from reagents or materials used during cell culture or EV isolation. For example, proteins or nucleic acid components originating from the culture media or ancillary reagents may be unintentionally introduced into EV preparations, potentially affecting product efficacy and safety (Stolk and Seifert 2015). Therefore, appropriate analytical tests are performed to exclude contamination by exogenous proteins, genetic material or other unintended substances.

2.2.5 | Purity

Purity is a key factor in ensuring the safety and effectiveness of stem cell-derived EVs. The purity of EVs refers to the relative proportion of EVs possessing specific characteristics. Impurities can be identified from multiple aspects, such as morphology, particle size distribution, functional components and chemical or biological composition (Webber and Clayton 2013; Wang, et al. 2024).

TEM can be used to determine if EVs are contaminated with cellular debris or other particles (Corona et al. 2023). The portion of EVs within a designated size range can also be regarded as one of the parameters of purity. Techniques such as NTA, TRPS and nano-flow cytometry can be used to assess the particle size distribution of EVs. EV products are expected to exhibit a uniform particle size distribution, avoiding excessive size variation or particles outside the designated range. Additionally, cellular debris or protein aggregates are detected to minimise the presence of non-EV components (Gandham et al. 2020).

During the separation and purification of EVs, protein contamination may occur. Cell membrane proteins or plasma proteins that have not been fully separated can mix with the EVs, affecting their purity and function. Immunoglobulins and coagulation factors may adhere to the EV surface, impacting their biological properties. For example, these proteins could alter the ability of EVs to bind to target cells or trigger unnecessary immune responses (Buzas 2023). Additionally, cell membrane proteins may interfere with EV recognition and endocytosis, reducing uptake efficiency by target cells (Zhang et al. 2019). Techniques such as Western blotting, nano-flow cytometry and mass spectrometry can confirm whether EVs are enriched with specific membrane proteins and detect contaminating proteins from various sources. Assessment of EVs purity also includes evaluation of exogenous proteins from the culture medium or external environment.

It is essential to assess whether nucleic acids are contaminated and evaluate their quality for functional studies. Nucleic acid contamination may arise from the cell's genome or from transcripts within the EVs. If these contaminants are not removed during purification, they can lead to genomic instability or other negative effects. DNA contamination can cause genomic instability, potentially leading to disease (Cai et al. 2013, 2016), while RNA contamination may interfere with the normal function of target cells or activate the host immune system, triggering unnecessary immune responses (Kouwaki et al. 2017; Williams, et al. 2023).

Moreover, some EVs may trigger immune responses due to proteins or nucleic acids attached to their outer membranes. Apart from detecting the contaminants themselves, their immunogenicity may also be evaluated to ensure the safety of EVs for clinical application and prevent immune rejection.

In addition to the exogenous impurities, membrane integrity is an important purity indicator related to the intrinsic source of EVs and is closely associated with their efficacy and safety. The EVs membrane plays crucial biological roles, including protecting the contents, interacting with target cells and participating in intercellular communication. Membrane integrity can be affected by various factors (such as pH, temperature, ionic strength), potentially leading to the leakage of EV contents or membrane rupture. Therefore, assessment of EV membrane integrity under different conditions, particularly during storage and transportation, is crucial to ensure the preservation of EVs function.

TEM provides detailed structural information about the EV membrane, including its integrity, thickness, and whether any damage or rupture is present. However, due to the limited number of EVs that can be observed, TEM cannot fully reflect the membrane integrity of entire batch of EVs. Several approaches have employed membrane-permeant enzymatic substrates to label EVs, such as carboxyfluorescein diacetate succinimidyl ester (CFSE), calcein acetoxymethyl ester (calcein-AM) or fluorescein diacetate (FDA). These molecules can be cleaved by esterases present inside cells and their secreted EVs. Once cleaved, the fluorescent signals from these molecules can reflect membrane integrity (Gray et al. 2015). By evaluating the permeability of these dyes, the integrity of the EV membrane can be assessed (Adamo et al. 2025). In addition, various other methods are also used to evaluate the membrane integrity of EVs from different perspectives and mechanisms. Regardless of the method used, thorough validation is required before its application in EV qual-

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

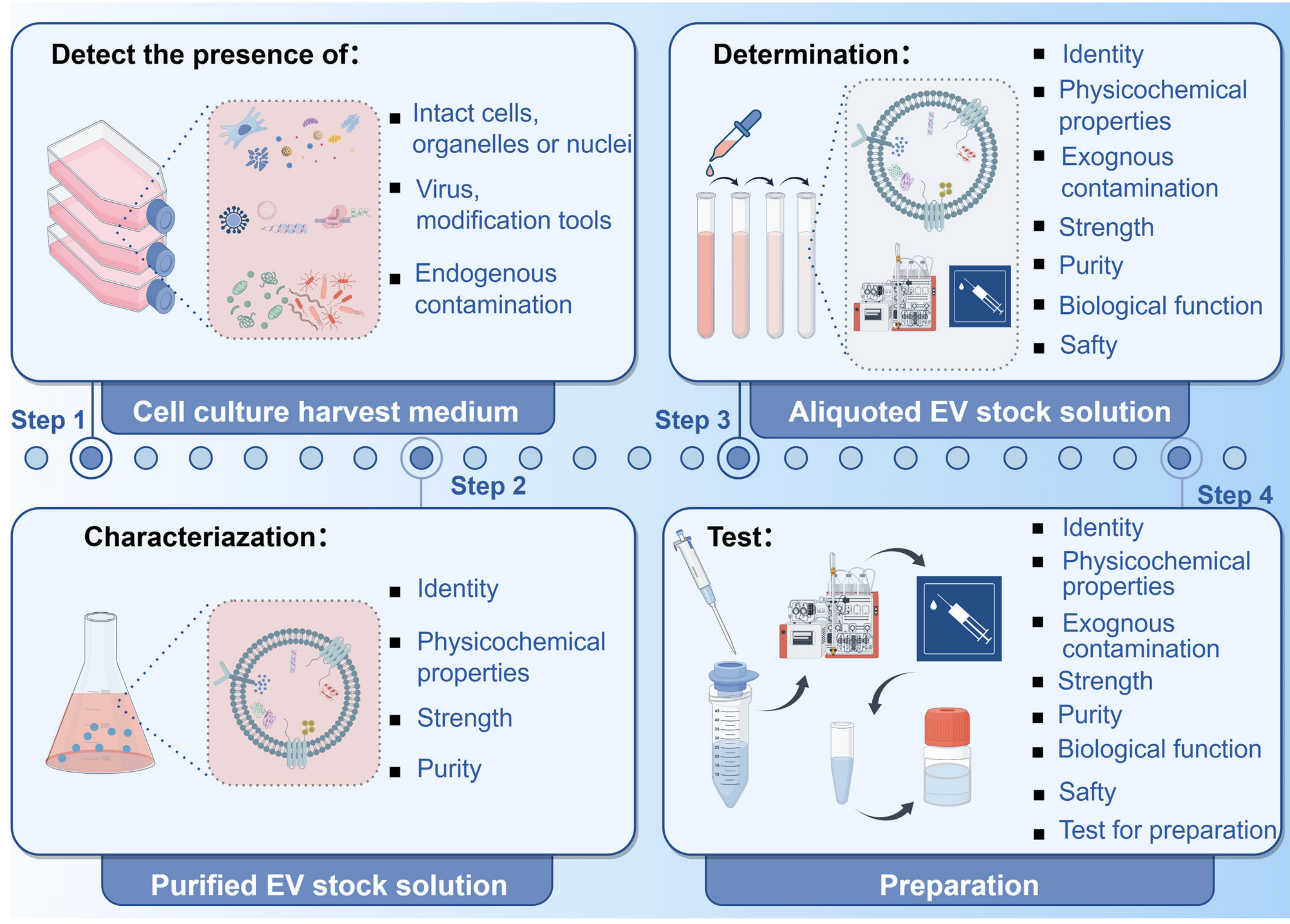

FIGURE 4 | Considerations for quality control strategy in the EV production process. If the cell culture supernatant is not immediately processed for subsequent purification but is instead stored frozen prior to EV isolation and purification, it is crucial to assess the presence of intact cells, organelles or nuclei. This evaluation is necessary to prevent the introduction of potential contaminants during the freeze-thaw process.

ity evaluation. Membrane integrity evaluation is not an isolated quality attribute; it can be combined with particle size distribution and active component analysis to assess the proportion of membrane-intact EVs within the EV particle size distribution range, or the proportion of EVs containing active components. These integrated approaches provide a more accurate count of effective EV numbers, which is beneficial for evaluating the safety and efficacy of EVs.

For PSC-derived EVs, obtaining a substantial quantity of EVs from conditioned medium requires culturing PSCs for longer periods than usual (Hsueh et al. 2023). During long-term cultivation, spontaneous differentiation may occur in PSCs. Additionally, EVs derived from incompletely or unintentionally differentiated cells (e.g., residual undifferentiated cells, progenitor cells, precursor cells) may contain tumourigenic factors, which could pose safety risks (Wang 2023). Strict evaluation is required to identify the presence of EVs from these non-target cells.

In summary, the establishment of a comprehensive strategy that includes morphology, particle size distribution, component analysis and membrane integrity enables the evaluation of EVs purity. This approach will enable effective quality control of production processes and ensure consistency across multiple batches of products, safeguarding the safety and efficacy of EV-based therapies.

2.2.6 | Biological Functions

EVs serve as important mediators of cellular communication and regulation in various physiological and pathological processes (Yates et al. 2022). Currently, stem cell-derived EVs are among the extensively studied due to their diverse biological functions, such as immune modulation, tissue repair and regeneration, angiogenesis and antioxidation (Rafieezadeh and Rafieezadeh 2024). In addition, EVs derived from different stem cell sources exhibit specific biological functions that are suited to therapeutic applications (Malik et al. 2025). Given the diverse EV biological functions of EVs, their roles can be categorised into two groups according to the intended therapeutic application. When targeting specific indications, the biological functions that are closely related to their therapeutic effects can be regarded as specific biological functions, whereas other biological functions are considered general biological functions. For example, recent studies have shown that EVs derived from human keratinocytes enhance the ability of melanocytes to generate dendrites and mature melanosomes, thereby promoting their efficient transfer. This effect can be considered a specific biological function of EVs

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 | Quality evaluation considerations for stem cell-derived EVs.[a]

Quality control contents of stem cell-derived EVs		Recommendations
1. Identity	Markers	It may be appropriate to determine negative and positive markers reflecting EV identity and source cell specificity.
	Source	The source of EVs, including the individual, cell type or tissue of origin, may be tested.
2. Physicochemical properties	Morphology	EVs are characterised by a lipid bilayer boundary.
	Particle size	EVs size range, average diameter, peak size and size distribution are preferably reported. If size distribution correlates with biological functions, a specific size range distribution standard is warranted.
	Zeta potential	It may be appropriate to ensure that EVs fall within a zeta potential range that maintains stability.
3. Adventitious contamination	Test for sterility	Methods specified in the ChP are recommended to be used. If a method different from the ChP requirements is applied, sufficient sensitivity is ensured to guarantee the safety of the EV product.
	Test for mycoplasmas	
	Test for specific contaminants	Testing may be based on potential contaminants that may be introduced during EV processing, such as specific viruses, small molecules or xenogeneic proteins derived from related reagents.
	Test for endotoxin	Methods specified in the ChP are recommended to be used.
4. Strength	Particle concentration	Total particle concentration, as well as the concentration of particles within the designated size range or with an intact lipid bilayer, may be reported.
	Total protein, nucleic acids or lipids, etc.	Results may be reported in terms of protein, nucleic acid or lipid content per a defined number of particles.
	Bioactive component	The parameter may be verified for its relevance to efficacy. Where feasible, the potency is determined based on intact membrane particles and/or particles within a designated size range. For safety considerations, it may also be appropriate to define the acceptable range of the total amount of bioactive components per defined number of particles, or the expression level or copy number of bioactive components in a single particle or a defined number of particles.
5. Purity	Membrane integrity	The proportion of particles with intact lipid bilayers is quantitatively assessed.
	Particles within size range	The proportion of particles, or intact membrane particles within the designated size range may be reported. Particles derived from excipients or protective agents in the preparations are also assessed.
	Rate of particles with bioactive component	The ratio of particles positive for bioactive components to total particles (or membrane-intact particles) may be reported.
	Non-target cells origin	For PSC differentiated cell-derived EVs, the proportion of EVs originating from undifferentiated, incompletely differentiated or abnormally differentiated cells may be considered. For gene-modified stem cell-derived EVs, consideration is also given to the proportion of EVs derived from non-modified cells.
6. Biological function	Mechanisms of action related components	Bioactive components—such as cytokines (including growth factors, immune factors, chemokines), nucleic acids, proteins and lipid—as well as activities related to the designated indications (e.g., angiogenesis, immune regulation and migration) may be analysed and reported as positivity rate, average content or potency, as evaluated using in vitro/in vivo models.

(Continues)

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 | (Continued)

Quality control contents of stem cell-derived EVs		Recommendations
	Intrinsic properties	Function-related properties, including membrane fluidity, protein corona components and/or zeta potential, be determined. Where feasible, the threshold range of these function-related properties may be analysed.
	General function	Given that most stem cell-derived EVs exhibit biological activities such as promoting migration and angiogenesis. These characteristics may be investigated as comprehensively as possible to provide traceable evidence for the future development of treatments for specific indications.
7. Safety	Carcinogenicity or tumourigenicity related factors	Risk factors—such as cytokines, miRNA, nucleic acids and lipids—may be quantitatively determined. If unavoidable risk factors are present, the threshold ranges that trigger these risks may be investigated and comprehensively validated using in vitro and in vivo models.
	Haemolysis/Coagulation	Testing in accordance with pharmacopoeia requirements may be performed. The effects of excipients or protective agents are taken into account.
	Immunogenicity	Related risk factors may be determined and validated using in vivo and/or in vitro models. The effects of excipients or protective agents should be evaluated.
8. Test for preparation	Preparation related characterizations	Sterility, mycoplasma contamination, endotoxin levels and species-related residues (such as bovine serum albumin) should be tested. In addition, parameters such as pH value, osmotic pressure, Zeta potential, particle size, particle number, membrane integrity, active ingredients and related materials (such as excipients or protective agents) are evaluated as appropriate. As excipients or protective agents may introduce interference in particle counting, assessment of intact membrane particles is considered particularly important for such preparations.

[a]The quality evaluation considerations for stem cell–derived EVs listed in the table are intended to serve as references. As product development advances and clinical research progresses, these considerations are expected to be continuously refined, supplemented and optimised in practice.

derived from human keratinocytes (Prospéri et al. 2024). General biological functions of EVs may serve as indispensable auxiliary functions to their overall therapeutic effectiveness. Therefore, it is essential to evaluate not only the specific biological functions of EVs but also as many of their general biological functions as possible. This approach enables a more comprehensive functional assessment and facilitates a deeper understanding of the underlying mechanisms of action.

From a quality control perspective, the following considerations may be taken into account when evaluating the biological activity of EVs. Firstly, appropriate in vitro quality evaluation methods are systematically established for different biological activities, such as promotion of cell proliferation, anti-apoptotic effects and other biological responses, to enable reliable assessment of EV biological activity. During the early stages of development, when efficacy-related quality control has not been clearly established, it is advisable to employ multiple complementary methods to analyse or quantify as many potential active components as possible. Systematic collection and curation of the resulting data enable the construction of a comprehensive product research database. This approach will facilitate the identification of quality control targets that are closely associated with efficacy through subsequent in-depth investigation. Once one or more efficacy-related targets have been identified, quantitative analytical methods are applied to establish robust quality control indicators for product efficacy, thereby ensuring better control of product quality and confirming product safety and effectiveness. Secondly, for biological functions associated with specific indications, the therapeutic efficacy and corresponding biological activities of EVs are rigorously assessed using both in vitro and in vivo models. Wherever feasible, quantitative potency assays may be considered to assess these indication-specific functions. Thirdly, relevant biomarkers for each target indication are assessed through both in both in vitro and in vivo studies to determine appropriate dose ranges. In parallel, the establishment of clear evaluation criteria, including total biomarker content per defined number of EVs and particle positivity rates, ensures batch-to-batch consistency, particularly regarding safety and efficacy in clinical applications. Finally, the stability of EVs is systematically assessed using efficacy-related biomarkers, with particular emphasis on potential changes under different storage conditions, including temperature, storage duration and repeated freeze-thaw cycles. The preservation of biological activity over time is verified to ensure reliability in clinical applications. Overall, research on EV biological functions is focused on understanding underlying mechanisms and develop-

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 | Considerations for candidate CQA attributes of EV-based therapeutic products.

CQA category	Candidate attributes[a]	Analytical methods[b]	Notes
Identity	Positive and negative markers	WB, Nano Flow cytometry, Fluorescence NTA	Negative markers are typically selected based on a risk-based approach and tailored to process- and product-specific impurities.
Physicochemical properties	Particle size distribution, Zeta potential	NTA, TRPS, Nano Flow cytometry, ELS	If particle size is associated with specific biological activity, this relationship typically requires rigorous validation.
Adventitious contamination	Endotoxin, sterility, mycoplasmas	LAL assay, culture-based methods; RMM; qPCR	Presence of these contaminations may indirectly affect the biological activity or potency of EV products.
Strength	Particle concentration	NTA, TRPS, Nano Flow cytometry	EV concentration is a dosing-related attribute rather than a potency-defining attribute.
Purity/Impurities	Membrane integrity; stability profile; HCPs; residual nucleic acid	Nano Flow cytometry, Fluorescence NTA, ELISA, qPCR	HCPs and residual nucleic acid are generally regarded as non-vesicular, free or process-related contaminants.
Potency	Bioactive ingredients, functional assays	Proteomics, nucleic acid analysis, lipidomics, in vitro assays or in vivo assays	Potency assays should be considered surrogate measures of biological activity rather than direct indicators of clinical outcome.
Specific tests for preparations	pH value, osmotic pressure, Zeta potential, appearance, nominal fill volume, Water Content/Residual Moisture, related materials	pH meter, Osmometer, ELS, Visual inspection, volumetric apparatus, Karl Fischer titrator, HPLC, MS	The selection and performance of tests for the preparation are dependent on the final dosage form of the EV preparation. For example, water content testing is applicable only to lyophilised preparations, whereas tests such as pH for lyophilised preparations shall be performed after reconstitution with the appropriate solvent in accordance with the product instructions for use.

Abbreviations: ELS, electrophoretic light scattering; HCPs, host cell proteins; HPLC, high-performance liquid chromatography; LAL, limulus amoebocyte lysate; MS, mass spectrometry; NTA, nanoparticle tracking analysis; RMM, rapid microbiological methods; TRPS, tunable resistive pulse sensing; WB, Western blotting.
[a]The candidate attributes listed here are general and broadly applicable to most stem cell–derived EV products. Developers may further refine or modify these attributes based on specific factors such as the manufacturing process, cell source and intended use.
[b]Other methods not listed here may also be adopted as appropriate.

ing the development of robust, standardised quality control strategies to ensure safety, efficacy and reproducibility in therapeutic use.

In addition to considering the substances carried by EVs, the intrinsic physicochemical characteristics of the EVs themselves may also influence their biological functions. Therefore, evaluation of factors such as membrane fluidity, protein corona formation and zeta potential is performed during quality research to ensure the consistency of the biological functions of EVs across different batches.

Membrane fluidity affects the ability of EVs to fuse with target cell membranes. If membrane fluidity is too low, fusion with target cells may be incomplete, thereby reducing the efficiency of cargo delivery. EVs with higher membrane fluidity are generally more effective at encapsulating and delivering their components, particularly lipophilic molecules, and can also adapt more readily to varying environmental conditions (such as changes in temperature or pH), thereby enhancing their stability and biocompatibility (Suga et al. 2021). It has been reported that freeze-thaw tolerance differs between cryopreserved synovial MSCs and human umbilical vein endothelial cells (HUVECs). Alterations in EV membrane fluidity contributed to pore-induced DMSO influx into the cytoplasm and increased reactive oxygen species production, resulting in greater cytotoxicity in HUVECs (Mizuno et al. 2022). Membrane fluidity can be investigated using techniques such as fluorescence recovery after photobleaching (FRAP), which monitors the diffusion behaviour of membrane proteins or lipids in real time; surface plasmon resonance, which indirectly assesses membrane fluidity by analysing membrane binding and dissociation processes; and single-molecule tracking, which evaluates membrane fluidity by monitoring the movement of individual molecules within the membrane (Verweij et al. 2021).

Protein corona is another inherent characteristic of EVs that significantly impacts their biological functions. It not only affects the

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

stability and structure of EVs but also regulates their interactions with target cells, immune evasion, signal transduction and their ability to deliver cargo (Önal Acet et al. 2024; Ragni and Taiana 2024). The protein corona primarily originates from components in the cell culture medium, particularly proteins such as albumin, immunoglobulins and fibrinogen (Panico et al. 2022). When EVs are exposed to biological fluids, either in vivo or in vitro, they interact with proteins in the fluid and absorb them onto their surface, forming a layer of various proteins known as the protein corona. The composition and characteristics of the protein corona are influenced by factors such as the source of the EVs, the type of biological fluid and environmental conditions (Dar et al. 2025). These components play important roles in the protein corona of EVs. The protein corona is not a 'contaminant', but rather a crucial modification layer that imparts specific biological identity and functions to EVs. Understanding and controlling the protein corona is essential for the development of efficient, safe and controllable EV-based therapeutic applications. The protein corona exerts several effects on the function of EVs (Liam-Or et al. 2024). Firstly, it may alter the immune characteristics of EVs, affecting how they are recognised and cleared by the immune system (Ding and Sun 2020). Some proteins may make EVs resemble autologous cells, reducing immune recognition and extending their circulation time in the body (Liam-Or et al. 2024), while other proteins may make EVs recognisable as foreign, triggering an immune response (Dietz et al. 2023). Secondly, the composition of the protein corona can directly affect the functions of EVs. For instance, some proteins adsorbed onto the EV surface may promote interactions with specific cell receptors, enhancing their affinity for and internalisation by target cells. In some cases, the protein corona may change the zeta potential or functionality of EVs, impacting biological processes such as cell-to-cell signalling, material transport and gene expression regulation (Wolf et al. 2022). Thirdly, the composition of the protein corona may determine how EVs interact with and are taken up by specific cells (da Costa Marques et al. 2023). Some proteins may facilitate entry into cells or uptake via specific receptor-mediated pathways. Finally, the formation of the protein corona increases the stability of EVs, protecting them from degradation in blood or bodily fluids. The protein corona shields EVs from enzymatic degradation in serum while helping maintain their structural integrity (Heidarzadeh et al. 2023). During the preparation of EVs, the composition of the protein corona may include exogenous proteins present in the medium, which poses a challenge for quality control. The potential impact of the protein corona may be underestimated in consistent quality assays across different EV batches. For example, some developers use the most abundant EV proteins as indicators of batch consistency to demonstrate process stability. While these proteins often appear stable across batches, the influence of protein corona components from the same cell culture system may be overlooked. Conversely, variability in the protein corona between batches may affect the consistency, safety and functionality of EVs. Quality control of the protein corona can be performed using techniques such as mass spectrometry or protein arrays to analyse its composition, as well as Western blotting or ELISA to detect specific proteins present in the protein corona. In addition, the impact of protein corona on biological functions may be evaluated by comparing primary EVs and protein corona depletion EVs (Wolf et al. 2022). Based on current scientific understanding and available methodologies, defining the protein corona as a CQA for EVs may be premature. However, careful consideration of its potential contribution to EV quality during development ensures process robustness, efficacy and safety.

As one of the intrinsic characteristics of EVs, zeta potential plays a crucial role in their function. In addition to its impact on EV stability, zeta potential can influence several other aspects of EV behaviour (Midekessa et al. 2020). Firstly, zeta potential affects the binding ability of EVs to target cells. Positively charged EVs are more likely to interact with negatively charged cell membranes, thereby promoting EV uptake, and vice versa (Dietz et al. 2023; Gul et al. 2024). Secondly, changes in zeta potential can influence the immunological properties of EVs (Dietz et al. 2023). EVs with a strong positive charge may evade immune recognition through specific immune evasion mechanisms. By modulating the zeta potential, the immune evasion capability, biodistribution and in vivo target delivery of EVs may be regulated (Rakshit Pal 2024). Thirdly, Zeta potential may indirectly affect the loading of cargo—such as RNA, proteins—onto EVs and influence their in vivo activities (Nakase et al. 2021). Adjusting the zeta potential may optimise their functionality in the biological environment (Gul et al. 2024). Overall, zeta potential is a key factor influencing EV function of EVs. It not only affects EV stability but also plays an important role in interactions with target cells, immune responses and cargo delivery. If zeta potential is identified as a factor affecting EV product quality, it may be considered as a CQA, in alignment with established regulatory precedents for nanomedicines, such as liposomes.

2.2.7 | Safety

As a therapeutic product, the safety risks associated with EVs can be broadly categorised into three aspects: the intrinsic components of EVs, risks arising from cell culture and EV preparation processes, and risks related to EV storage and handling. These safety concerns primarily include carcinogenicity, cytotoxicity, immunogenicity and contamination. A systematic and comprehensive evaluation of these factors is essential to minimise safety risks and maximise the therapeutic efficacy of EV-based interventions.

2.2.7.1 | Carcinogenicity or Tumourigenicity. EVs are non-replicative vesicles, and most researchers consider EVs to be a safe therapeutic product. However, stem cell-derived EV still may carry a risk of carcinogenesis or tumour promotion. Dysregulation of tumour suppressor genes, apoptosis-related genes and angiogenesis-related genes is closely associated with tumour occurrence, proliferation and development. Studies have found that expression of certain miRNAs may lead to tumour initiation, development, metastasis, angiogenesis and drug resistance. For instance, umbilical cord MSC-derived EVs carrying miR-21 significantly promote corneal epithelial cell proliferation, migration and wound healing in rats (Liu et al. 2022), while another study also demonstrates their ability to protect β-cells from hypoxia-induced apoptosis by alleviating endoplasmic reticulum stress and inhibiting p38 MAPK signalling (Chen et al. 2020). However, miR-21 also exhibits carcinogenic properties, and increasing evidence suggests that miR-21 serves as a tumour biomarker (Li and Tie 2024). Mechanistically, miR-21 primarily downregulates

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

PTEN expression, thereby activating the PI3K/Akt signalling pathway during cancer progression. Overexpression of miR-21 suppresses apoptosis, enhances glycolysis to promote tumour growth, induces epithelial-mesenchymal transition (EMT), increases the expression of MMP-2 and MMP-9, and consequently facilitates tumour metastasis (Hashemi et al. 2023). Recent studies have shown that small EVs secreted by cardiac stromal cells following myocardial infarction carry multiple tumourigenic factors, which can accelerate tumour growth (Caller et al. 2024). In addition, accumulating evidence links EV-associated proteins, miRNAs, RNAs and lipids to carcinogenesis or tumourigenesis, underscoring the necessity for stringent control of EV components (Jiang et al. 2022; Patel et al. 2025). Therefore, several strategies may be considered to mitigate the carcinogenic or tumourigenic risks of EV-based therapeutics. First, establishing a comprehensive database of EV components that are highly associated with carcinogenicity or tumourigenicity could facilitate risk assessment and component screening. Secondly, limiting the abundance of high-risk components in EVs may help reduce potential safety concerns. For components that exhibit both therapeutic efficacy and carcinogenic or tumourigenic potential, careful characterisation and regulation of their expression levels in EVs would be advisable, ensuring that the effective dose remains well below the risk-associated threshold. In addition, it may be important to evaluate whether the disease-specific microenvironment in vivo enhances the activity or synergistic effects of high-risk components. Finally, routine monitoring of multiple batches of EV-producing cells and the associated variability in EV components could contribute to improved quality control. A thorough assessment of carcinogenic or tumourigenic risks within the permissible fluctuation ranges of critical components would provide a solid foundation for maintaining product quality consistency and ensuring clinical safety.

2.2.7.2 | Coagulation and Haemolysis. Although EVs play an important role in physiological processes, their dysregulated activity under certain pathological conditions may raise safety concerns, particularly in thrombosis (coagulation promotion) and red blood cell rupture (haemolysis).

Under normal physiological conditions, EVs contribute to haemostasis by promoting coagulation. However, in specific pathological contexts, they may trigger aberrant thrombotic events. EVs promote excessive thrombus formation by enhancing the activation of coagulation factors and platelet aggregation (He and Wu 2023). For instance, in chronic diseases such as cardiovascular disease, cancer and diabetes, EVs may increase coagulation activity, leading to inappropriate thrombus formation within blood vessels (He and Wu 2023). This hypercoagulable state can result in life-threatening complications, including stroke, myocardial infarction or deep vein thrombosis. Mechanistically, EVs expose negatively charged phospholipids, such as phosphatidylserine (PS), which provide a catalytic surface for coagulation reactions and accelerate the coagulation cascade (Tripisciano et al. 2017). Consequently, EVs may act as contributors to thrombotic disorders in susceptible patients. In addition, certain EV populations may carry fibrinolysis inhibitors, potentially delaying thrombus dissolution and further exacerbating thrombosis risk, thereby compromising the safety of thrombus resolution (Shiotsu et al. 2024).

Haemolysis refers to the rupture of red blood cells, resulting in the release of haemoglobin into the bloodstream. Although EVs generally contribute to maintaining extracellular homeostasis, they may also be implicated in haemolysis under certain conditions (Weber et al. 2025). Interaction between EVs and red blood cell membranes can induce membrane damage or exacerbate haemolytic processes through the release of cytotoxic molecules. The physicochemical stability of EVs directly influences their safety in circulation. If EVs are unstable or inadequately cleared, they may exacerbate damage to red blood cells or the vascular endothelium, thereby increasing the risks of both haemolysis and coagulation. Although the involvement of EVs in coagulation and haemolysis is physiologically relevant, their potential safety implications in clinical applications and disease progression warrant further investigation and careful control.

2.2.7.3 | Immunogenicity. EVs can induce immune responses through multiple mechanisms, including immunogenic reactions, immune activation, immune rejection, immune evasion, and, under certain conditions, autoimmune reactions. The immunomodulatory effects of EVs are complex and involve multiple immune pathways. Key mechanisms underlying EV-induced immune response include immune recognition, activation of immune effector cells and alterations in immune tolerance.

EVs may contain molecules that are recognised by the immune system as 'non-self', thereby eliciting an immune response (Chen et al. 2019). Proteins, lipids and RNA species located on or within EVs may be perceived as foreign by the host immune system, particularly when their cellular origin differs from that of the recipient. Consequently, EVs may be cleared by immune cells such as macrophages, dendritic cells or B cells. This immune-mediated clearance may compromise the stability and therapeutic efficacy of EVs in vivo. For example, EVs may carry immunogenic proteins, including major histocompatibility complex (MHC) class I molecules, tumour-associated antigens, which can activate antigen-presenting cells, such as dendritic cells, thereby stimulating T cell responses and inducing antigen-specific immunity. EVs can also activate the host immune system through specific molecules they carry, such as RNA or proteins. For example, EVs can carry immune-active molecules, including cytokines, chemokines and lipid mediators, which can activate immune cells (Kumari et al. 2024). MicroRNAs (miRNAs), long non-coding RNAs (lncRNAs), or mRNAs carried by EVs can trigger immune responses via immune receptors such as Toll-like receptors (TLRs) or RIG-I receptors. In addition, the protein corona, cell-derived surface markers or membrane receptors may lead to recognition and clearance of EVs by the host immune system. Conversely, some EVs can evade immune system recognition through mechanisms such as protein corona formation, alterations in surface charge or other surface modifications (Dietz et al. 2023). Although immune evasion may reduce immune clearance and enhance therapeutic efficacy, it can also negatively affect the effectiveness of EV-based therapies in certain contexts. For instance, EVs containing exogenous RNA have been reported to act as damage associated molecular patterns, thereby triggering innate immune response mechanisms (Essola et al. 2024). EVs containing specific proteins may enhance immune system's reaction, such as inducing interleukin-6 (IL-6) production (Kitai et al. 2017). Moreover, EVs enriched in heat shock proteins (e.g.,

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

HSP60 and HSP90) expressing highly immunogenic molecules, including MHC-I, MHC II, CD40, CD86, RANTES and IL-1β, may stimulate adaptive immune responses (Chen et al. 2006). In some situations, EVs may trigger autoimmune responses (Lu et al. 2021). This can occur when EVs carry self-antigens or components released from damaged cells, leading the immune system to attack the body's own tissues. In certain autoimmune diseases, EVs may contribute to the breakdown of immune tolerance by transporting self-antigens. For example, in systemic lupus erythematosus (SLE), EVs may carry components derived from damaged cells, thereby triggering immune responses against these self-components and exacerbate autoimmune reactions (Fortin et al. 2016).

To explore the potential risk of immune responses associated with EVs, analytical approaches such as mass spectrometry or Western blot could be used to examine whether EVs carry or overexpress specific receptors or ligands (e.g., TLR, MHC-I/II molecules) that might contribute to immune activation. EVs-induced immune cell responses could also be explored using in vitro cell models, such as co-cultures systems with human dendritic cells or macrophages. In addition, cytotoxicity assays (e.g., MTT, CCK-8) could preliminary insights into the potential toxicity of EVs to immune cells (e.g., T cells, B cells, and macrophages), as highly cytotoxic EVs may be associated with localised or systemic immune responses. Animal models and in vitro experiments could further help to explore whether EVs are associated with specific immune responses or with modulation of T cell activity under different immune conditions. Finally, RT-qPCR or RNA-sequencing could be used to examine changes in gene expression in recipient cells following EVs treatment, particularly in genes related to immune responses, including immune suppression or activation.

Although EVs hold broad therapeutic potential, careful consideration of their immunological characteristics is essential in therapeutic applications to minimise the risk of adverse immune responses. A comprehensive understanding of the interactions between EVs and the immune system is essential for the development of safer and more effective EV-based therapeutic products.

2.2.7.4 | Residues. During the preparation of EVs, various chemicals (e.g., solvents, reagents) may be used. If these chemicals are not adequately removed, they can result in chemical contamination. Residual solvents may cause cytotoxicity, while certain reagents could trigger immune responses or interfere with EV function, such as inhibiting their binding to or endocytosis by target cells (Barragán-Martínez et al. 2012). Therefore, chemicals associated with higher risks, including cytotoxicity or tumourigenicity, are taken into careful consideration during cell culture as well as EV isolation and purification processes. The residual levels of these components are assessed in the final EV product to ensure compliance with the requirements for therapeutic applications.

For genetically modified stem cell-derived EVs, it may be advisable not only to evaluate the EVs populations lacking the intended components but also to assess the potential presence of unintended exogenous genes or genetic material, in order to reduce the risk of gene contamination (Kawamura et al. 2017). For PSC-derived EVs, EV containing relatively high levels of specific transcription factors may warrant additional evaluation. In addition, for PSC-differentiated cell-derived EVs, consideration could be given to assessing whether residual differentiation-inducing reagents are present in the EV preparations, particularly those known to pose tumourigenic or pro-tumourigenic risks. Similarly, for immortalised cell-derived EVs, it may be appropriate to evaluate whether they contain transcription factors or other bioactive components associated with tumourigenicity or pro-tumourigenicity.

2.2.7.5 | Special Considerations for Cell Types. The quality requirements for different types of stem cell-derived EVs may vary depending on differences in their compositions and biological activities, which could influence the applicable quality standards. In general, stability and immunogenicity may be considered key aspects to ensure that stem cell-derived EVs do not elicit excessive immune responses. It may also be important to assess whether these EVs have the potential to induce uncontrolled cell proliferation or undesirable immune reactions.

MSC-derived EVs are commonly explored for applications in tissue repair and immune modulation; therefore, maintaining anti-inflammatory, immunoregulatory and tissue repair–related activities may be desirable. Quality control strategies for these EVs could focus on the stability and bioactivity of critical components, such as cytokines, miRNAs and relevant molecular or surface markers.

PSC-derived EVs play an important role in regenerative medicine applications. However, EVs from PSCs may pose a tumourigenic risk due to the presence of components that could promote excessive cell proliferation or abnormal differentiation. Accordingly, quality control for these EVs involves the identification and mitigation of potential tumourigenic risks. Monitoring the stability and activity of stem cell-related transcription factors and specific miRNAs, particularly with respect to their roles in cell proliferation, is important. In addition, preventing elevated levels of residual pluripotency markers in EV preparations is critical to minimising potential tumourigenic risks.

Genetically modified stem cell-derived EVs, such as those produced using CRISPR/Cas9 or other gene-editing technologies, may carry specific genetic modifications. Therefore, stringent quality monitoring is necessary to ensure that these modifications do not result in genetic instability or unintended side effects. The presence and expression levels of genetically modified components in EVs helps prevent the introduction of potential mutations or unsafe gene expressions. In addition, genetic modification may alter the intrinsic biological properties of EVs. Therefore, comprehensive investigations are necessary to determine whether such modifications affect the expression of EV-associated risk factors, induce the upregulation of risk-related molecules, or influence immunogenicity. Furthermore, it is essential to elucidate whether genetic modification alters the inherent biological functions of EVs and to clarify whether these functions act synergistically or antagonistically with the engineered therapeutic functions in specific disease indications. Ultimately, the use of in vivo models enables assessment of the net outcome of EVs derived from genetically modified cells within the relevant disease microenvironment, thereby determining their

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

actual therapeutic efficacy and safety for particular indications, such as cancer.

Immortalised stem cells, which are genetically modified to possess unlimited proliferative capacity, may produce EVs that carry components associated with cell proliferation. Quality control for these EVs therefore involves ensuring they do not contain factors that could promote tumour formation, such as proteins involved in cell cycle regulation, proliferation or apoptosis.

2.2.8 | Stability

The stability of EVs is closely associated with their efficacy and safety, and may encompass structural, compositional, functional and safety-related aspects. The membrane structure of EVs may be affected by the external conditions encountered during processes such as isolation, purification and storage, potentially leading to changes in morphology and size, as well as membrane rupture or particle aggregation. In addition, EVs may be influenced by environmental factors, including temperature, pH, ion strength or concentration gradients across the membrane, which could affect cargo integrity, membrane stability and overall function. Alterations in EV structure and composition may consequently influence their biological activity and safety. For example, membrane damage could result in the leakage or loss of bioactive molecules, leading to reduced biological efficacy. Moreover, exposure of immune-active components following membrane disruption might be associated with unintended immune responses. EVs may also undergo oxidation damage or degradation during storage or transportation, further affecting their stability and biological activity.

Lipid components of the EV membrane could be susceptible to oxidation, potentially compromising membrane integrity or function, while EV-associated proteins and RNA may degrade under suboptimal or prolonged storage conditions, thereby diminishing their biological effects. Accordingly, appropriate quality control measures may be considered at critical stages of the EV lifecycle, including isolation, purification, packaging, storage and transportation. Monitoring EV stability throughout the entire process could be important to support their safety and efficacy in clinical applications.

2.2.9 | Differences Between the Aforementioned Quality Evaluation Strategies and MISEV Guidelines

As discussed above, the MISEV guidelines are widely recognised as among the most authoritative and comprehensive guidance documents in the EV research field to date. They have played a pivotal role in promoting standardised terminology and improving experimental design, thereby enhancing scientific rigour and reproducibility. The latest version, MISEV2023 (Welsh et al. 2024), incorporates a range of emerging technologies and offers valuable direction for the continued refinement of EV basic research. In comparison, the quality evaluation strategies described in this paper seek to explore how CQAs might be systematically established to help ensure product safety and efficacy, particularly in the context of regulatory submissions to agencies such as the NMPA. From this complementary and translational perspective, certain distinctions may arise between the framework proposed here and the EV analysis and characterization approaches outlined in the MISEV guidelines.

2.2.9.1 | Differences in Purpose. When ISEV released the first version of MISEV in 2014 (Lötvall et al. 2014), its primary aim was to standardise terminology and propose baseline experimental validation criteria for vesicle-related research, with the goal of improving rigour and reproducibility. Within this framework, a central question was how to demonstrate that the entities under investigation were indeed EVs, and that the observed biological effects could be attributed specifically to EVs rather than to non-EV components. Such research standards are unquestionably foundational for the advancement of EV-based therapeutics. However, from the standpoint of regulatory science and product development, additional dimensions may merit consideration. The quality evaluation strategies outlined in this paper are therefore intended to further address issues related to safety and efficacy assurance in the context of pharmaceutical development and regulatory evaluation.

2.2.9.2 | Further Specification of Certain Requirements. Using 'Identity' as an example, MISEV recommends the use of broadly accepted positive and negative protein markers (e.g., tetraspanins CD9, CD63, CD81 and selected cytoplasmic proteins) to support vesicle characterization. While these markers are highly valuable for confirming EV-associated features, they are generally shared across multiple cell types and may not fully distinguish donor origin, tissue source or genetic modification status. For therapeutic product development, it may therefore be appropriate to consider incorporating additional source-specific markers that are closely linked to defined cell origins and manufacturing processes. In this context, it may not only be important to demonstrate that the product consists of EVs, but also to provide evidence of derivation from specific cell types (e.g., well-characterised MSCs or iPSCs). Where feasible, aligning such markers with functional relevance to potency could further strengthen product characterisation.

2.2.9.3 | Significant Expansion in Certain Evaluation Dimensions. With respect to in vitro and in vivo studies, MISEV2023 places substantial emphasis on appropriate experimental controls. In a translational and clinical development setting, however, additional evaluation dimensions may warrant systematic consideration. For example, safety assessment strategies intended to support clinical application might incorporate more detailed quality control parameters, including tumourigenicity, immunogenicity, coagulation or haemolysis risk, and the detection of exogenous residues. Although these aspects extend beyond the primary scope of research-oriented guidelines, they may become particularly relevant as EV products progress toward clinical use.

2.2.9.4 | Evaluation Throughout the Entire Manufacturing Process. Using cells as an illustrative example, MISEV2023 recommends reporting cell sources and culture conditions. In the context of therapeutic product manufacturing, more extensive characterisation of the production cell bank may be advisable, as it represents an early and critical element of EV manufacturing and quality control. Notably, cell lines deemed suitable for cell

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

therapy applications may not necessarily be optimal for EV production. Accordingly, additional evaluations—such as EV secretion yield, phenotypic stability and passage consistency—could be considered to support manufacturing robustness and batch-to-batch consistency.

2.2.9.5 | Greater Alignment With Pharmaceutical Definitions. MISEV2023 tends to use particle numbers or total protein content as quantitative metrics. From a pharmaceutical perspective, however, particle count alone may not be sufficient as a release specification and may need to be interpreted in conjunction with functional bioactivity data. In this regard, the present paper discusses the potential incorporation of pharmaceutical concepts such as strength and potency. Specifically, Strength may be defined as the quantity of defined active components (e.g., selected miRNAs or proteins) per unit particle number. Potency, in turn, would ideally be established through validated biological activity assays that are mechanistically and clinically relevant to the intended indication.

2.2.9.6 | Different Strategies for Analytical Method Selection. In addition to the previously mentioned detection methods, MISEV2023 introduces numerous new technologies and proposes the use of orthogonal methods (multiple methods validating each other) for characterising EV critical quality attributes. We, however, distinguish between the Research and Development (R&D) phase and the Release Testing phase.

R&D Phase: Consistent with MISEV recommendations, high-throughput, comprehensive and orthogonal methods (e.g., RNA sequencing) may be particularly valuable for in-depth characterisation and mechanistic understanding.

Release Phase: Greater emphasis may be placed on efficiency, robustness, reproducibility and validated assays suitable for routine batch release. For instance, targeted qPCR assays might be considered as practical alternatives to sequencing methods, which may be less feasible for routine quality control due to time and cost constraints.

In summary, MISEV may be viewed primarily as a broadly applicable research standard aimed at strengthening the rigour and reproducibility of EV basic research. The quality evaluation strategies discussed in this paper are intended to complement this foundation by addressing considerations that may become increasingly important in industrial translation and clinical development, particularly in light of regulatory expectations and the practical challenges associated with the development and commercialisation of EV-based therapeuti c products.

2.3 | Considerations on EVs Preparation

When considering the preservation of EV preparations, the selection of an appropriate storage method is critical for maintaining cargo stability and preserving biological activity for clinical applications. In the quality control of EV preparations, in addition to general quality attributes (such as purity, stability, size distribution, etc.), application-specific requirements may also need to be carefully monitored to support efficacy and safety across different use scenarios.

Currently, EV preparations are commonly available in two main forms: lyophilised (freeze-dried) and non-lyophilised (liquid) (Shen et al. 2025). Available evidence suggests that EVs may undergo structural and functional alterations when exposed to suboptimal storage conditions, including temperature fluctuations and repeated freeze-thaw cycles (Ahmadian et al. 2024). To improve stability, EVs preparations may be formulated with additional excipients intended to preserve membrane integrity and functional activity. For long-term storage, various buffer additives, such as bovine serum albumin (Abraham and Goel 2025), sucrose (Walker et al. 2022), human serum albumin plus trehalose (Görgens et al. 2022) and PLGA-PEG (Khoshravan et al. 2022) have been explored for their potential to enhance EVs stability. Compatibility of these additives with EVs and the absence of adverse effects on biological function or safety are essential considerations. For EV formulations, particle size uniformity and size may represent important factors influencing biological activity and therapeutic efficacy. Therefore, it may be important to confirm that the formulation remains within an acceptable size range, avoiding excessive aggregation, oversized particles or precipitation. In addition, potential interactions between additives EV surface receptors or proteins are assessed, as such interactions could disrupt the lipid bilayer or lead to the loss of surface-associated proteins, thereby reducing efficacy. Finally, consideration may also be given to screening for species-specific viruses that could potentially be introduced through animal- or human-derived additives.

EVs may be susceptible to aggregation, precipitation or functional loss during long-term storage as a result of environmental factors such as temperature and pH variations. Therefore, it may be important to define and optimise appropriate storage conditions to help maintain the stability of EV preparations during transportation and prolonged storage. For lyophilised EV formulations, particular attention could be given to ensuring that the freeze-drying process does not compromise EV integrity or biological activity, and that the EVs are able to recover their original functionality upon reconstitution. In addition, the physical appearance of the lyophilised preparations may also be examined to confirm uniformity and the absence of visible precipitation, aggregation or insoluble particles.

EV preparations may also require predefined release characteristics to support the controlled and effective delivery of functional molecules. In vitro release studies could be employed to characterise release profiles and to evaluate the stability and sustained activity of EV-associated bioactive components.

Immunogenicity represents an important consideration for EV preparations and may need to be assessed to reduce the risk of unintended immune activation or adverse immune responses. Accordingly, immunogenicity testing could be considered an integral component of quality control. Furthermore, the compatibility of EV preparations with target cells or tissues may be evaluated, including their ability to enter cells and deliver functional cargos without inducing cytotoxicity or adverse cellular responses. Finally, comprehensive chemical characterization of all components within EV preparations including EVs them-

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

selves, residual chemicals and formulation excipients, could be performed to verify component identity, purity and consistency, while excluding unnecessary or potentially harmful substances.

Consistency between production batches is an important consideration for EV preparations. Compliance of each batch with predefined quality requirements supports the reliability and safety of therapeutic applications. Batch consistency may encompass parameters such as particle size distribution, strength, purity, biological activity, potency and formulation stability. For clinical applications of EV preparations, determination of an appropriate dose may require animal studies or preclinical investigations to ensure that EVs exert the intended biological effects within a suitable dosage range. Biological activity assays may be necessary to confirm that EV preparations retain their specific functional properties during treatment, such as immune regulation, cell repair and anti-inflammatory activity. To support batch-to-batch consistency, each step of the manufacturing process may need to be carefully monitored. This includes quality oversight of EV isolation, purification, packaging, lyophilisation and reconstitution processes, which collectively contribute to the overall consistency and quality of the final EV preparation.

During long-term storage of EV formulations, degradation may be unavoidable. Therefore, stability and quality studies could include characterisation and control of degradation products formed during storage. Analytical techniques such as high-performance liquid chromatography (HPLC) or mass spectrometry may be applied to compare EV samples stored at different time points, enabling the detection of protein fragments, new components or other molecular degradation products. Such analyses could help confirm the occurrence of degradation, identify degradation products and evaluate the extent of degradation. In addition, periodic monitoring of parameters such as purity (e.g., membrane integrity) and biological activity may provide indirect indicators of EV stability over time.

EV preparations may need to be dissolved or dispersed in water or other solvents prior to use; therefore, evaluating their solubility and dispersibility in different solvents may be important to ensure formulation suitability for therapeutic applications. Furthermore, stability studies under varying temperature, humidity and light conditions could be conducted to assess whether EV preparations retain their biological activity during transportation and storage.

The selection of packaging material for EV preparations represents another important consideration. Packaging provides protection against moisture, air and potential contaminants. Materials such as glass containers or aluminium-sealed vials are commonly used; their compatibility with EVs may need to be evaluated to ensure the absence of adverse interactions. Following packaging, container closure integrity testing could be performed to verify that the packaging remains impermeable to external contamination during transportation and storage. Maintaining sealing integrity may be crucial to prevent environmental factors from compromising the quality of EV preparations.

Overall, quality control of EV preparations may involve not only the characteristics of the EVs themselves but also consideration of stability, dosage, immunogenicity, purity and batch consistency throughout the manufacturing process. Systematic monitoring of these parameters could help support the safety and efficacy of EV preparations intended for therapeutic use.

2.4 | Considerations on Methods

Quality evaluation of EV-based products is inherently multifaceted. During product and process development, complementary analytical platforms may be employed to enhance understanding of specific EV attributes. However, once an attribute is established as a CQA, selection of analytical methods for in-process control and release testing is determined by fitness for purpose, suitability for validation, interpretability for quality decision-making, reproducibility and lifecycle suitability. In accordance with the ICH Q8–Q12 framework, exploratory and advanced methodologies may be appropriate during development to support CQA identification, whereas release testing methods are required to be scientifically justified, validated in accordance with ICH Q2, and directly linked to safety and efficacy as outlined in ICH Q6B. Therefore, analytical tool selection may follow a lifecycle-based, risk-driven approach, prioritising robustness and relevance over technological complexity. Ultimately, CQA evaluation relies on the most appropriate method rather than the most advanced one.

In EV quality control, the use of multiple detection methods to evaluate the same parameter may be appropriate; however, careful method selection depends on specific analytical objectives and the characteristics of each technique.

Firstly, applying orthogonal methods to measure the same parameter may help address methodological limitations from different perspectives and enable cross-validation of results, thereby improving data reliability. Secondly, the choice of detection methods depends on whether the analytical goal is qualitative or quantitative. Qualitative and quantitative analyses serve distinct purposes and have different requirements; therefore, method selection is aligned with the intended detection objective. In practice, these approaches are often complementary. Qualitative analyses can be used to confirm the presence and composition of EVs, whereas quantitative analyses provide numerical data to assess whether EV products (or EV preparations) meet predefined standards and specifications. The selection of detection methods may also depend on the intended application of the EVs. For instance, when the objective is to characterise basic EV properties, qualitative techniques such as Western blotting may suffice. In contrast, when precise determination of the concentration or activity of a specific EV subpopulation is required, quantitative methods such as nano-flow cytometry may be more appropriate. Combining multiple analytical techniques may therefore provide a more comprehensive assessment of EV quality. As an example, protein quantification can be performed using colourimetric methods, Western blotting and mass spectrometry to evaluate total protein content, identify specific proteins and quantify target protein levels, respectively. Thirdly, different methods vary in sensitivity and specificity. The use of multiple techniques may help broaden the detectable range and mitigate the limitations associated with individual methods. For low-abundance targets or applications with high sensitivity requirements, advanced quantitative techniques such as mass spectrometry or real-

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

time quantitative PCR may be preferable. For example, EV size distribution can be assessed using NTA, TRPS or nano-flow cytometry, each of which differs in particle size detection range, optimal concentration requirements and resolution. A combined approach may enable more complete coverage of the EV size distribution, including particles outside the primary target size range. Fourthly, some methods may provide incomplete information for certain parameters, whereas complementary techniques can help fill these gaps. For example, conventional NTA and TRPS allow measurement of EV size distribution but do not directly provide information on surface protein expression. In such cases, protein labelling or immunodetection approaches, such as fluorescence-based NTA or nano-flow cytometry, may be used to supplement the analysis. Finally, analytical methods differ in cost, time requirements and operational complexity. In EV quality control, method selection may therefore need to consider the specific objectives and requirements at different stages, such as quality research and release testing. During the quality research stage, the focus is typically on comprehensive characterisation of EV properties, including composition, function, stability and related attributes. This stage may require more sensitive, detailed and comprehensive analytical techniques to support R&D activities. In contrast, release testing aims to confirm that EV products meet predefined quality specifications prior to clinical use or market release, with an emphasis on efficiency, accuracy and reproducibility. Accordingly, methods used at this stage are often high throughput, standardised and relatively simple to operate, providing rapid and reliable quantitative data to support batch consistency and product safety. For instance, while high-throughput RNA sequencing may be informative for comprehensive RNA profiling during research, it may be costly and time-consuming for routine testing. In release testing, targeted real-time quantitative PCR for specific RNAs may represent a more practical, efficient and cost-effective alternative.

In summary, the use of multiple analytical methods to assess the same parameter can be advantageous in EV quality control; however, the selection of an appropriate combination of methods is based on specific analytical needs and the inherent characteristics of each technique to ensure both accuracy and comprehensive evaluation of results.

2.5 | Summary

Based on the key quality attributes of stem cell-derived EV products discussed above, a proposed quality evaluation strategy is summarised in Figure 4. This figure illustrates the major quality control points throughout the EV production process, together with corresponding quality control elements, which are provided as reference examples. During the development of EV-based therapeutic products, additional control points may need to be established according to product type, potential risk factors, and process-specific considerations. For example, if cell culture supernatants are not immediately processed for downstream purification and are instead stored under frozen conditions prior to EV isolation and purification, it may be important to assess the presence of intact cells, cellular organelles or nuclei. Such evaluation could help minimise the risk of introducing contaminants associated with freeze-thaw-induced cell damage. Furthermore, for each quality control element, developers may select appropriate analytical and testing strategies based on process characteristics, potential risks and the intended application of the material at each critical control point, as illustrated in the figure.

The considerations outlined above primarily focus on the quality evaluation of stem cell-derived EVs, including identification, purity, impurities, biological activity and safety. These aspects are closely linked to various stages of production, ranging from the cell culture harvest medium to the final product. Table 3 presents detailed recommendations corresponding to each quality evaluation content. It is important to emphasise that, when evaluating the quality of EV samples at different stages, alteration of the condition or morphology of the samples is avoided during the testing process. Unnecessary operations, such as the pH or ion strength of the solution, or filtering samples prior to testing to remove aggregated particles, are generally avoided during quality evaluation. Such operations may distort the intrinsic characteristics of the EV samples, potentially leading to evaluation results that do not accurately or consistently reflect the final efficacy and safety of the product.

To enhance the practical applicability of the proposed framework, a summary table of representative CQAs commonly applicable to EV-based therapeutic products is provided in Table 4. This table summarises relatively generalisable CQAs that are less sensitive to variations in cell source, manufacturing process and intended use, and is intended to serve as a conceptual reference rather than prescriptive guidance. The analytical methods are presented as indicative examples rather than prescriptive standards. Accordingly, the table presented here should not be interpreted as a fixed or universally applicable standard. In particular, attributes related to specific molecular cargo (e.g., proteins, RNAs) or mechanism-of-action–dependent potency may vary substantially between products and therefore require case-by-case evaluation. The omission of such product-specific CQAs from the summary table reflects an intentional effort to avoid overgeneralization and to maintain alignment with a risk-based and science-driven approach. For specific products, additional attributes may be introduced or existing ones adjusted based on the principles outlined in this manuscript.

3 | Conclusion

Stem cell-derived EVs have demonstrated significant potential as therapeutic products in the fields of regenerative medicine in recent years. However, their clinical translation continues to face several challenges. Large-scale production and standardisation of stem cell-derived EVs remain difficult. Variations in cell sources and culture systems, as well as differences in isolation and purification processes, may lead to discrepancies in the purity, biological activity and stability of EVs across different batches. Such variability could affect the reproducibility and safety of their clinical applications.

Like other therapeutic products, quality evaluations play a central role in the development and application of stem cell-derived EVs. From the R&D stage through clinical trials, systematic quality evaluation is important at each stage to support the safety

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and potential effectiveness of EV-based products. During the development of EV-based therapies, studies on product safety and efficacy may be designed to be comprehensive and well designed, with sufficient data accumulation to provide traceable references and supporting evidence. Efficacy evaluations may benefit from the inclusion of clearly defined quantitative indicators to more comprehensively assess the biological activity and therapeutic potential of EVs. The establishment of quality strategies and control measures is often guided by risk management principles that take into account factors such as cell types, production process and intended clinical application. Such approaches may help support the consistency, stability and safety of EV research products. As research in this field continues to advance, it is anticipated that more standardised production and quality control frameworks for EVs will be gradually developed. These may include guidance on cell culture conditions, EV isolation and purification methods, as well as storage and transportation practices, to facilitate clinical translation. It is also worth noting that non-cell-based therapies do not inherently ensure safety, as biological effects observed in animal models may not fully translate to humans, particularly with respect to dosing and toxicity. Therefore, prior to first-in-human studies, the advanced development of appropriate evaluation and risk mitigation strategies is generally considered advisable.

In conclusion, stem cell-derived EVs represent an emerging class of therapeutic candidates with considerable potential. Ongoing advances in EV biology, manufacturing technologies and analytical methodologies are expected to further elucidate key determinants of EV quality and function. The considerations proposed in this article are not intended to serve as official regulatory guidelines but are provided for reference and discussion among researchers and developers engaged in EV-related product development. As these efforts continue, more refined and harmonised approaches to EV characterisation and quality assessment may gradually emerge, fostering a deeper understanding of EV-based mechanisms and informing their future development in therapeutic research.

Author Contributions

Tao Na: conceptualization, funding acquisition, writing – original draft, writing – review and editing, formal analysis. **Kehua Zhang**: conceptualization, writing – original draft, writing – review and editing. **Libo Zhao**: writing – review and editing, writing – original draft. **Xuanbao Yao**: writing – review and editing. **Chongfeng Xu**: funding acquisition, writing – review and editing. **Yuan Zhang**: writing – review and editing. **Lan Wang**: funding acquisition, writing – review and editing, supervision. **Miao Xu**: writing – review and editing, supervision. **Hui Zhang**: supervision, funding acquisition. **Shufang Meng**: conceptualization, writing – original draft, funding acquisition, writing – review and editing, supervision.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (grant numbers: 2021YFA1101601 and 2024YFA1107301), the State Key Laboratory of Drug Regulatory Science (grant number: 2024SKLDRS0208). Table of contents image drawn with assistance from Figdraw.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability Statement

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

References

Abraham, M., and S. Goel. 2025. “Species-Specific Optimisation of Cryopreservation Media for Goat and Buffalo Adipose-Derived Mesenchymal Stem Cells.” *Cryobiology* 118: 105211.

Adamo, G., S. Picciotto, P. Gargano, et al. 2025. “DetectEV: A Functional Enzymatic Assay to Assess Integrity and Bioactivity of Extracellular Vesicles.” *Journal of Extracellular Vesicles* 14: e70030.

Ahmadian, S., N. Jafari, A. Tamadon, A. Ghaffarzadeh, R. Rahbarghazi, and M. Mahdipour. 2024. “Different Storage and Freezing Protocols for Extracellular Vesicles: A Systematic Review.” *Stem Cell Research & Therapy* 15: 453.

Arab, T., E. R. Mallick, Y. Huang, et al. 2021. “Characterization of Extracellular Vesicles and Synthetic Nanoparticles With Four Orthogonal Single-Particle Analysis Platforms.” *Journal of Extracellular Vesicles* 10: e12079.

Babula, A., I. Siemińska, and M. Baj-Krzyworzeka. 2023. “Isolation of Low Endotoxin Content Extracellular Vesicles Derived From Cancer Cell Lines.” *Journal of Visualized Experiments*. 192: e65062. https://doi.org/10.3791/65062.

Badosa, C., M. Roldán, J. Fernández-Irigoyen, E. Santamaria, and C. Jimenez-Mallebrera. 2023. “Proteomic and Functional Characterisation of Extracellular Vesicles From Collagen VI Deficient Human Fibroblasts Reveals a Role in Cell Motility.” *Scientific Reports* 13: 14622.

Barragán-Martínez, C., C. A. Speck-Hernández, G. Montoya-Ortiz, R. D. Mantilla, J.-M. Anaya, and A. Rojas-Villarraga. 2012. “Organic Solvents as Risk Factor for Autoimmune Diseases: A Systematic Review and Meta-Analysis.” *PLoS ONE* 7: e51506.

Bordanaba-Florit, G., F. Royo, S. G. Kruglik, and J. M. Falcón-Pérez. 2021. “Using Single-Vesicle Technologies to Unravel the Heterogeneity of Extracellular Vesicles.” *Nature Protocols* 16: 3163–3185.

Buzas, E. I. 2023. “The Roles of Extracellular Vesicles in the Immune System.” *Nature Reviews Immunology* 23: 236–250.

Cai, J., Y. Han, H. Ren, et al. 2013. “Extracellular Vesicle-Mediated Transfer of Donor Genomic DNA to Recipient Cells Is a Novel Mechanism for Genetic Influence Between Cells.” *Journal of Molecular Cell Biology* 5: 227–238.

Cai, J., G. Wu, P. A. Jose, and C. Zeng. 2016. “Functional Transferred DNA Within Extracellular Vesicles.” *Experimental Cell Research* 349: 179–183.

Caller, T., I. Rotem, O. Shaihov-Teper, et al. 2024. “Small Extracellular Vesicles From Infarcted and Failing Heart Accelerate Tumor Growth.” *Circulation* 149: 1729–1748.

Chandler, W. L., W. Yeung, and J. F. Tait. 2011. “A New Microparticle Size Calibration Standard for Use in Measuring Smaller Microparticles Using a New Flow Cytometer.” *Journal of Thrombosis and Haemostasis* 9: 1216–1224.

Chen, J., J. Chen, Y. Cheng, et al. 2020. “Mesenchymal Stem Cell-Derived Exosomes Protect Beta Cells Against Hypoxia-Induced Apoptosis via miR-21 by Alleviating ER Stress and Inhibiting p38 MAPK Phosphorylation.” *Stem Cell Research & Therapy* 11: 97.

Chen, W., J. Wang, C. Shao, et al. 2006. “Efficient Induction of Antitumor T Cell Immunity by Exosomes Derived From Heat-Shocked Lymphoma Cells.” *European Journal of Immunology* 36: 1598–1607.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Chen, Z., A. T. Larregina, and A. E. Morelli. 2019. "Impact of Extracellular Vesicles on Innate Immunity." *Current Opinion in Organ Transplantation* 24: 670–678.

Chen, Z., L. Luo, T. Ye, et al. 2024. "Identification of Specific Markers for Human Pluripotent Stem Cell-Derived Small Extracellular Vesicles." *Journal of Extracellular Vesicles* 13: e12409.

Choi, J. K., E. C. Mbanefo, M. K. Yadav, et al. 2025. "Interleukin 35-Producing B Cells Prolong the Survival of GVHD Mice by Secreting Exosomes With Membrane-Bound IL-35 and Upregulating PD-1/LAG-3 Checkpoint Proteins." *Theranostics* 15: 3610–3626.

Chow, F. W., and R. M. Morphew. 2023. "Extracellular Vesicles in Microbes, Pathogens, and Infectious Diseases." *International Journal of Molecular Sciences* 24: 10686.

Cocucci, E., and J. Meldolesi. 2015. "Ectosomes and Exosomes: Shedding the Confusion Between Extracellular Vesicles." *Trends in Cell Biology* 25: 364–372.

Comfort, N., K. Cai, T. R. Bloomquist, M. D. Strait, A. W. Ferrante Jr., and A. A. Baccarelli. 2021. "Nanoparticle Tracking Analysis for the Quantification and Size Determination of Extracellular Vesicles." *Journal of Visualized Experiments*. 169: e62447. https://doi.org/10.3791/62447.

Corona, M. L., I. Hurbain, G. Raposo, et al. 2023. "Characterization of Extracellular Vesicles by Transmission Electron Microscopy and Immunolabeling Electron Microscopy." *Methods in Molecular Biology* 2668: 33–43.

Cvjetkovic, A., S. C. Jang, B. Konečná, et al. 2016. "Detailed Analysis of Protein Topology of Extracellular Vesicles-Evidence of Unconventional Membrane Protein Orientation." *Scientific Reports* 6: 36338.

da Costa Marques, R., N. Hüppe, K. R. Speth, et al. 2023. "Proteomics Reveals Time-Dependent Protein Corona Changes in the Intracellular Pathway." *Acta Biomaterialia* 172: 355–368.

Dar, A. I., S. Randhawa, M. Verma, T. C. Saini, and A. Acharya. 2025. "Debugging the Dynamics of Protein Corona: Formation, Composition, Challenges, and Applications at the Nano-Bio Interface." *Advances in Colloid and Interface Science* 342: 103535.

Dietz, L., J. Oberländer, A. Mateos-Maroto, et al. 2023. "Uptake of Extracellular Vesicles Into Immune Cells Is Enhanced by the Protein Corona." *Journal of Extracellular Vesicles* 12: e12399.

Ding, T., and J. Sun. 2020. "Mechanistic Understanding of Cell Recognition and Immune Reaction via CR1/CR3 by HAP- and SiO(2)-NPs." *BioMed Research International* 2020: 7474807.

Essola, J. M., M. Zhang, H. Yang, et al. 2024. "Exosome Regulation of Immune Response Mechanism: Pros and Cons in Immunotherapy." *Bioactive Materials* 32: 124–146.

Farkas, N., J. A. Kramar, A. R. Montoro Bustos, et al. 2025. "Derivation of Particle Number Concentration From the Size Distribution: Theory and Applications." *Analytical Chemistry* 97: 10999–11006.

Fordjour, F. K., C. Guo, Y. Ai, G. G. Daaboul, and S. J. Gould. 2022. "A Shared, Stochastic Pathway Mediates Exosome Protein Budding Along Plasma and Endosome Membranes." *Journal of Biological Chemistry* 298: 102394.

Fortin, P. R., N. Cloutier, V. Bissonnette, et al. 2016. "Distinct Subtypes of Microparticle-Containing Immune Complexes Are Associated With Disease Activity, Damage, and Carotid Intima-Media Thickness in Systemic Lupus Erythematosus." *Journal of Rheumatology* 43: 2019–2025.

Fyfe, J., I. Casari, M. Manfredi, and M. Falasca. 2023. "Role of Lipid Signalling in Extracellular Vesicles-Mediated Cell-to-Cell Communication." *Cytokine & Growth Factor Reviews* 73: 20–26.

Gandham, S., X. Su, J. Wood, et al. 2020. "Technologies and Standardization in Research on Extracellular Vesicles." *Trends in Biotechnology* 38: 1066–1098.

Ghadami, S., and K. Dellinger. 2023. "The Lipid Composition of Extracellular Vesicles: Applications in Diagnostics and Therapeutic Delivery." *Frontiers in Molecular Biosciences* 10: 1198044.

Giannessi, F., A. Aiello, F. Franchi, Z. A. Percario, and E. Affabris. 2020. "The Role of Extracellular Vesicles as Allies of HIV, HCV and SARS Viruses." *Viruses* 12: 571.

Görgens, A., G. Corso, D. W. Hagey, et al. 2022. "Identification of Storage Conditions Stabilizing Extracellular Vesicles Preparations." *Journal of Extracellular Vesicles* 11: e12238.

Gray, W. D., A. J. Mitchell, and C. D. Searles. 2015. "An Accurate, Precise Method for General Labeling of Extracellular Vesicles." *MethodsX* 2: 360–367.

Gudbergsson, J. M., K. B. Johnsen, M. N. Skov, and M. Duroux. 2016. "Systematic Review of Factors Influencing Extracellular Vesicle Yield From Cell Cultures." *Cytotechnology* 68: 579–592.

Gul, R., H. Bashir, M. Sarfraz, et al. 2024. "Human Plasma Derived Exosomes: Impact of Active and Passive Drug Loading Approaches on Drug Delivery." *Saudi Pharmaceutical Journal* 32: 102096.

Gupta, D., A. M. Zickler, and S. El Andaloussi. 2021. "Dosing Extracellular Vesicles." *Advanced Drug Delivery Reviews* 178: 113961.

Hashemi, M., M. S. A. Mirdamadi, Y. Talebi, et al. 2023. "Pre-Clinical and Clinical Importance of miR-21 in Human Cancers: Tumorigenesis, Therapy Response, Delivery Approaches and Targeting Agents." *Pharmacological Research* 187: 106568.

He, Y., and Q. Wu. 2023. "The Effect of Extracellular Vesicles on Thrombosis." *Journal of Cardiovascular Translational Research* 16: 682–697.

Heidarzadeh, M., A. Zarebkohan, R. Rahbarghazi, and E. Sokullu. 2023. "Protein Corona and Exosomes: New Challenges and Prospects." *Cell Communication and Signaling* 21: 64.

Herrmann, I. K., M. J. A. Wood, and G. Fuhrmann. 2021. "Extracellular Vesicles as a Next-Generation Drug Delivery Platform." *Nature Nanotechnology* 16: 748–759.

Hsueh, Y.-H., W. Buddhakosai, P. N. Le, et al. 2023. "Therapeutic Effect of Induced Pluripotent Stem Cell-Derived Extracellular Vesicles in an In Vitro and In Vivo Osteoarthritis Model." *Journal of Orthopaedic Translation* 38: 141–155.

Huang, C., Y. R. Neupane, X. C. Lim, et al. 2021. "Extracellular Vesicles in Cardiovascular Disease." *Advances in Clinical Chemistry* 103: 47–95.

Huang, Z., J. Li, J. Wo, et al. 2025. "Intranasal Delivery of Brain-Derived Neurotrophic Factor (BDNF)-Loaded Small Extracellular Vesicles for Treating Acute Spinal Cord Injury in Rats and Monkeys." *Journal of Extracellular Vesicles* 14: e70066.

Hur, Y. H., S. Feng, K. F. Wilson, R. A. Cerione, and M. A. Antonyak. 2021. "Embryonic Stem Cell-Derived Extracellular Vesicles Maintain ESC Stemness by Activating FAK." *Developmental Cell* 56: 277–291.e6.

Iovine, J. C., S. M. Claypool, and N. N. Alder. 2021. "Mitochondrial Compartmentalization: Emerging Themes in Structure and Function." *Trends in Biochemical Sciences* 46: 902–917.

Jeppesen, D. K., A. M. Fenix, J. L. Franklin, et al. 2019. "Reassessment of Exosome Composition." *Cell* 177: 428–445.e18.

Jeyaram, A., and S. M. Jay. 2017. "Preservation and Storage Stability of Extracellular Vesicles for Therapeutic Applications." *AAPS Journal* 20: 1.

Jiang, Q., X.-P. Tan, C.-H. Zhang, et al. 2022. "Non-Coding RNAs of Extracellular Vesicles: Key Players in Organ-Specific Metastasis and Clinical Implications." *Cancers* 14: 5693.

Jiao, Y.-R., K.-X. Chen, X. Tang, et al. 2024. "Exosomes Derived From Mesenchymal Stem Cells in Diabetes and Diabetic Complications." *Cell Death & Disease* 15: 271.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Kalluri, R., and V. S. LeBleu. 2020. "The Biology, Function, and Biomedical Applications of Exosomes." *Science* 367: eaau6977.

Kannan, S., S. Gokul Krishna, P. K. Gupta, and U. K. Kolkundkar. 2024. "Advantages of Pooling of Human Bone Marrow-Derived Mesenchymal Stromal Cells From Different Donors Versus Single-Donor MSCs." *Scientific Reports* 14: 12654.

Kawamura, Y., Y. Yamamoto, T.-A. Sato, and T. Ochiya. 2017. "Extracellular Vesicles as Trans-Genomic Agents: Emerging Roles in Disease and Evolution." *Cancer Science* 108: 824–830.

Khoshravan, L., M. Dadashpour, M. Hashemi, and N. Zarghami. 2022. "Design and Development of Nanostructured Co Delivery of Artemisinin and Chrysin for Targeting hTERT Gene Expression in Breast Cancer Cell Line: Possible Clinical Application in Cancer Treatment." *Asian Pacific Journal of Cancer Prevention* 23: 919–927.

Kitai, Y., T. Kawasaki, T. Sueyoshi, et al. 2017. "DNA-Containing Exosomes Derived From Cancer Cells Treated With Topotecan Activate a STING-Dependent Pathway and Reinforce Antitumor Immunity." *Journal of Immunology* 198: 1649–1659.

Kmiotek-Wasylewska, K., A. Łabędź-Masłowska, S. Bobis-Wozowicz, et al. 2024. "Induced Pluripotent Stem Cell-Derived Extracellular Vesicles Enriched With miR-126 Induce Proangiogenic Properties and Promote Repair of Ischemic Tissue." *FASEB Journal* 38: e23415.

Koritzinsky, E. H., J. M. Street, R. A. Star, and P. S. T. Yuen. 2017. "Quantification of Exosomes." *Journal of Cellular Physiology* 232: 1587–1590.

Kou, M., L. Huang, J. Yang, et al. 2022. "Mesenchymal Stem Cell-Derived Extracellular Vesicles for Immunomodulation and Regeneration: A Next Generation Therapeutic Tool?" *Cell Death & Disease* 13: 580.

Kouwaki, T., M. Okamoto, H. Tsukamoto, Y. Fukushima, and H. Oshiumi. 2017. "Extracellular Vesicles Deliver Host and Virus RNA and Regulate Innate Immune Response." *International Journal of Molecular Sciences* 18: 666.

Kugeratski, F. G., K. Hodge, S. Lilla, et al. 2021. "Quantitative Proteomics Identifies the Core Proteome of Exosomes With Syntenin-1 as the Highest Abundant Protein and a Putative Universal Biomarker." *Nature Cell Biology* 23: 631–641.

Kukaj, D., S. Niebert, C. Biehl, U. Reichart, C. Schueler, and J. Burk. 2025. "No Simple Way to Averaging Out: Pooled Mesenchymal Stromal Cells Do Not Reflect Average Donor Characteristics." *Regenerative Therapy* 30: 849–859.

Kumar, M. A., S. K. Baba, H. Q. Sadida, et al. 2024. "Extracellular Vesicles as Tools and Targets in Therapy for Diseases." *Signal Transduction and Targeted Therapy* 9: 27.

Kumari, P., S. S. Wright, and V. A. Rathinam. 2024. "Role of Extracellular Vesicles in Immunity and Host Defense." *Immunological Investigations* 53: 10–25.

Lai, R. C., and S. K. Lim. 2019. "Membrane Lipids Define Small Extracellular Vesicle Subtypes Secreted by Mesenchymal Stromal Cells." *Journal of Lipid Research* 60: 318–322.

Lee, H., X. He, T. Le, J. M. Carnino, and Y. Jin. 2020. "Single-Step RT-qPCR for Detection of Extracellular Vesicle microRNAs In Vivo: A Time- and Cost-Effective Method." *American Journal of Physiology-Lung Cellular and Molecular Physiology* 318: L742–L749-l749.

Le Saux, S., A. Aubert-Pouëssel, K. E. Mohamed, et al. 2021. "Interest of Extracellular Vesicles in Regards to Lipid Nanoparticle Based Systems for Intracellular Protein Delivery." *Advanced Drug Delivery Reviews* 176: 113837.

Levy, D., S. N. Abadchi, N. Shababi, et al. 2023. "Induced Pluripotent Stem Cell-Derived Extracellular Vesicles Promote Wound Repair in a Diabetic Mouse Model via an Anti-Inflammatory Immunomodulatory Mechanism." *Advanced Healthcare Materials* 12: e2300879.

Li, H., and X. J. Tie. 2024. "Exploring Research Progress in Studying Serum Exosomal miRNA-21 as a Molecular Diagnostic Marker for Breast Cancer." *Clinical and Translational Oncology* 26: 2166–2171.

Li, L., Y. Wang, Y. Xu, et al. 2024. "ROS-Scavenging Lipid-Based Liquid Crystalline as a Favorable Stem Cell Extracellular Vesicles Delivery Vector to Promote Wound Healing." *Journal of Controlled Release* 371: 298–312.

Li, Q., Q. Liu, S. Li, et al. 2025. "Golgi-Derived Extracellular Vesicle Production Induced by SARS-CoV-2 Envelope Protein." *Apoptosis* 30: 197–209.

Liam-Or, R., F. N. Faruqu, A. Walters, et al. 2024. "Cellular Uptake and In Vivo Distribution of Mesenchymal-Stem-Cell-Derived Extracellular Vesicles Are Protein Corona Dependent." *Nature Nanotechnology* 19: 846–855.

Liao, K., J. Yu, A. Akhmerov, et al. 2025. "Long Noncoding RNA BCYRN1 Promotes Cardioprotection by Enhancing Human and Murine Regulatory T Cell Dynamics." *Journal of Clinical Investigation* 135: e179262.

Liu, X., X. Li, G. Wu, et al. 2022. "Umbilical Cord Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Deliver miR-21 to Promote Corneal Epithelial Wound Healing Through PTEN/PI3K/Akt Pathway." *Stem Cells International* 2022: 1–15.

Lötvall, J., A. F. Hill, F. Hochberg, et al. 2014. "Minimal Experimental Requirements for Definition of Extracellular Vesicles and Their Functions: A Position Statement From the International Society for Extracellular Vesicles." *Journal of Extracellular Vesicles* 3: 26913.

Lou, P., X. Zhou, Y. Zhang, et al. 2025. "Harnessing Tissue-Derived Mitochondria-Rich Extracellular Vesicles (Ti-mitoEVs) to Boost Mitochondrial Biogenesis for Regenerative Medicine." *Science Advances* 11: eadt1318.

Lu, J.-Q., D. Liu, Y.-Z. Qou, et al. 2024. "Research and Suggestions on the Scope and Classification of Advanced Therapy Medicinal Product in China." China Food & Drug Administration Magazine 5: 10–25.

Lu, M., E. DiBernardo, E. Parks, H. Fox, S.-Y. Zheng, and E. Wayne. 2021. "The Role of Extracellular Vesicles in the Pathogenesis and Treatment of Autoimmune Disorders." *Frontiers in Immunology* 12: 566299.

Luo, L., Z. Chen, J. Yuan, et al. 2025. "Identification of a Specific Marker Panel for Human Mesenchymal Stem Cell-Derived Small Extracellular Vesicles." *Interdisciplinary Medicine* 3: e70065.

Malik, S. Z. A., Y. Muhilan, F. Nordin, et al. 2025. "Stem Cell Derived Exosome Trilogy: An Epic Comparison of Human MSCs, ESCs and iPSCs." *Stem Cell Research & Therapy* 16: 318.

McConnell, R. E., M. Youniss, B. Gnanasambandam, P. Shah, W. Zhang, and J. D. Finn. 2022. "Transfection Reagent Artefact Likely Accounts for Some Reports of Extracellular Vesicle Function." *Journal of Extracellular Vesicles* 11: e12253.

Mehdizadeh, S., M. Mamaghani, S. Hassanikia, Y. Pilehvar, and Y. N. Ertas. 2025. "Exosome-Powered Neuropharmaceutics: Unlocking the Blood-Brain Barrier for Next-Gen Therapies." *Journal of Nanobiotechnology* 23: 329.

Mendivil-Alvarado, H., A. T. Limon-Miro, E. Carvajal-Millan, et al. 2023. "Extracellular Vesicles and Their Zeta Potential as Future Markers Associated With Nutrition and Molecular Biomarkers in Breast Cancer." *International Journal of Molecular Sciences* 24: 6810.

Miceli, R. T., T.-Y. Chen, Y. Nose, et al. 2024. "Extracellular Vesicles, RNA Sequencing, and Bioinformatic Analyses: Challenges, Solutions, and Recommendations." *Journal of Extracellular Vesicles* 13: e70005.

Midekessa, G., K. Godakumara, J. Ord, et al. 2020. "Zeta Potential of Extracellular Vesicles: Toward Understanding the Attributes That Determine Colloidal Stability." *ACS Omega* 5: 16701–16710.

Mizenko, R. R., M. Feaver, B. T. Bozkurt, et al. 2024. "A Critical Systematic Review of Extracellular Vesicle Clinical Trials." *Journal of Extracellular Vesicles* 13: e12510.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Mizuno, M., T. Matsuzaki, N. Ozeki, et al. 2022. "Cell Membrane Fluidity and ROS Resistance Define DMSO Tolerance of Cryopreserved Synovial MSCs and HUVECs." *Stem Cell Research & Therapy* 13: 177.

Moulin, C., M. J. F. Crupi, C. S. Ilkow, J. C. Bell, and S. Boulton. 2023. "Extracellular Vesicles and Viruses: Two Intertwined Entities." *International Journal of Molecular Sciences* 24: 1036.

Nakase, I., N. Ueno, M. Matsuzawa, et al. 2021. "Environmental pH Stress Influences Cellular Secretion and Uptake of Extracellular Vesicles." *FEBS Open Bio* 11: 753–767.

Nguyen, V. V. T., J. A. Welsh, T. Tertel, et al. 2024. "Inter-Laboratory Multiplex Bead-Based Surface Protein Profiling of MSC-Derived EV Preparations Identifies MSC-EV Surface Marker Signatures." *Journal of Extracellular Vesicles* 13: e12463.

Noble, J. M., L. M. Roberts, N. Vidavsky, et al. 2020. "Direct Comparison of Optical and Electron Microscopy Methods for Structural Characterization of Extracellular Vesicles." *Journal of Structural Biology* 210: 107474.

Nolte-'t Hoen, E., T. Cremer, R. C. Gallo, and L. B. Margolis. 2016. "Extracellular Vesicles and Viruses: Are They Close Relatives?" *Proceedings of the National Academy of Sciences* 113: 9155–9161.

Önal Acet, B., D. Gül, R. H. Stauber, et al. 2024. "A Review for Uncovering the "Protein-Nanoparticle Alliance": Implications of the Protein Corona for Biomedical Applications." *Nanomaterials (Basel)* 14: 823.

Panico, S., S. Capolla, S. Bozzer, G. Toffoli, M. Dal Bo, and P. Macor. 2022. "Biological Features of Nanoparticles: Protein Corona Formation and Interaction With the Immune System." *Pharmaceutics* 14: 2605.

Patel, B., S. Gaikwad, and S. Prasad. 2025. "Exploring the Significance of Extracellular Vesicles: Key Players in Advancing Cancer and Possible Theranostic Tools." *Cancer Pathogenesis and Therapy* 3: 109–119.

Pegtel, D. M., and S. J. Gould. 2019. "Exosomes." *Annual Review of Biochemistry* 88: 487–514.

Pham, C. V., S. Midge, H. Barua, et al. 2021. "Bovine Extracellular Vesicles Contaminate Human Extracellular Vesicles Produced in Cell Culture Conditioned Medium When 'Exosome-Depleted Serum' is Utilised." *Archives of Biochemistry and Biophysics* 708: 108963.

Prieto-Vila, M., Y. Yoshioka, N. Kuriyama, et al. 2024. "Adult Cardiomyocytes-Derived EVs for the Treatment of Cardiac Fibrosis." *Journal of Extracellular Vesicles* 13: e12461.

Prospéri, M.-T., C. Giordano, M. Gomez-Duro, et al. 2024. "Extracellular Vesicles Released by Keratinocytes Regulate Melanosome Maturation, Melanocyte Dendricity, and Pigment Transfer." *Proceedings of the National Academy of Sciences* 121: e2321323121.

Quah, B. J. C., and H. C. O'Neill. 2007. "Mycoplasma Contaminants Present in Exosome Preparations Induce Polyclonal B Cell Responses." *Journal of Leukocyte Biology* 82: 1070–1082.

Raab-Traub, N., and D. P. Dittmer. 2017. "Viral Effects on the Content and Function of Extracellular Vesicles." *Nature Reviews Microbiology* 15: 559–572.

Rafieezadeh, D. 2024. "Extracellular Vesicles and Their Therapeutic Applications: A Review Article (part1)." *International Journal of Physiology, Pathophysiology and Pharmacology* 16: 1–9.

Ragni, E., and M. Taiana. 2024. "Mesenchymal Stromal Cells-Extracellular Vesicles: Protein Corona as a Camouflage Mechanism?" *Extracellular Vesicles and Circulating Nucleic Acids* 5: 722–727.

Rahnama, M., M. Heidari, Z. Poursalehi, and A. Golchin. 2024. "Global Trends of Exosomes Application in Clinical Trials: A Scoping Review." *Stem Cell Reviews and Reports* 20: 2165–2193.

Raj, D. A. A., I. Fiume, G. Capasso, and G. Pocsfalvi. 2012. "A Multiplex Quantitative Proteomics Strategy for Protein Biomarker Studies in Urinary Exosomes." *Kidney International* 81: 1263–1272.

Rakshit, T., and S. Pal. 2024. "Extracellular Vesicles for Drug Delivery and Theranostics In Vivo." *Journal of the American Chemical Society Au* 4: 318–327.

Ramalingam, M., S. Jang, J. Hwang, H.-H. Cho, B. C. Kim, and H.-S. Jeong. 2025. "Neural-Induced Human Adipose Tissue-Derived Stem Cell Secretome Exerts Neuroprotection Against Rotenone-Induced Parkinson's Disease in Rats." *Stem Cell Research & Therapy* 16: 193.

Riazifar, M., E. J. Pone, J. Lötvall, and W. Zhao. 2017. "Stem Cell Extracellular Vesicles: Extended Messages of Regeneration." *Annual Review of Pharmacology and Toxicology* 57: 125–154.

Rohde, E., K. Pachler, and M. Gimona. 2019. "Manufacturing and Characterization of Extracellular Vesicles From Umbilical Cord-Derived Mesenchymal Stromal Cells for Clinical Testing." *Cytotherapy* 21: 581–592.

Sanghvi, S., D. Sridharan, P. Evans, et al. 2025. "Functional Large-Conductance Calcium and Voltage-Gated Potassium Channels in Extracellular Vesicles Act as Gatekeepers of Structural and Functional Integrity." *Nature Communications* 16: 42.

Schroeder, A., O. Mueller, S. Stocker, et al. 2006. "The RIN: An RNA Integrity Number for Assigning Integrity Values to RNA Measurements." *BMC Molecular Biology* 7: 3.

Shang, J., and X. Gao. 2014. "Nanoparticle Counting: Towards Accurate Determination of the Molar Concentration." *Chemical Society Reviews* 43: 7267–7278.

Shao, H., H. Im, C. M. Castro, X. Breakefield, R. Weissleder, and H. Lee. 2018. "New Technologies for Analysis of Extracellular Vesicles." *Chemical Reviews* 118: 1917–1950.

Shen, J., Y. He, J. Deng, et al. 2025. "Storage Stability of Exosomes in Different Buffers With/Without Lyophilization." *Journal of Drug Delivery Science and Technology* 112: 107278.

Shiotsu, H., D. Sueta, S. Shinriki, et al. 2024. "miR-4485-5p in Large Extracellular Vesicles as a New Potent Biomarker for Diagnosis of Deep Vein Thrombosis." *Laboratory Medicine International* 3: 95–107.

Sivanantham, A., H. Lee, and Y. Jin. 2022. "Direct Detection of Extracellular Vesicle miRNAs Using a Single-Step RT-qPCR Assay." *Methods in Molecular Biology* 2504: 137–145.

Skotland, T., A. Llorente, and K. Sandvig. 2023. "Lipids in Extracellular Vesicles: What Can Be Learned About Membrane Structure and Function?" *Cold Spring Harbor Perspectives in Biology* 15: a041415.

Skotland, T., K. Sagini, K. Sandvig, and A. Llorente. 2020. "An Emerging Focus on Lipids in Extracellular Vesicles." *Advanced Drug Delivery Reviews* 159: 308–321.

Stolk, M., and M. Seifert. 2015. "Protein Contaminations Impact Quantification and Functional Analysis of Extracellular Vesicle Preparations From Mesenchymal Stromal Cells." *Journal of Stem Cells and Regenerative Medicine* 11: 44–47.

Suga, K., D. Matsui, N. Watanabe, Y. Okamoto, and H. Umakoshi. 2021. "Insight Into the Exosomal Membrane: From Viewpoints of Membrane Fluidity and Polarity." *Langmuir* 37: 11195–11202.

Takakura, Y., R. Hanayama, K. Akiyoshi, et al. 2024. "Quality and Safety Considerations for Therapeutic Products Based on Extracellular Vesicles." *Pharmaceutical Research* 41: 1573–1594.

Tanudisastro, H. A., I. W. Deveson, H. Dashnow, and D. G. MacArthur. 2024. "Sequencing and Characterizing Short Tandem Repeats in the Human Genome." *Nature Reviews Genetics* 25: 460–475.

Tesovnik, T., B. Jenko Bizjan, R. Šket, M. Debeljak, T. Battelino, and J. Kovač. 2021. "Technological Approaches in the Analysis of Extracellular Vesicle Nucleotide Sequences." *Frontiers in Bioengineering and Biotechnology* 9: 787551.

Tian, S., X. Zhou, M. Zhang, et al. 2022. "Mesenchymal Stem Cell-Derived Exosomes Protect Against Liver Fibrosis via Delivering miR-148a to Target KLF6/STAT3 Pathway in Macrophages." *Stem Cell Research & Therapy* 13: 330.

Tian, Y., M. Gong, Y. Hu, et al. 2020. "Quality and Efficiency Assessment of Six Extracellular Vesicle Isolation Methods by Nano-Flow Cytometry." *Journal of Extracellular Vesicles* 9: 1697028.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Tripisciano, C., R. Weiss, T. Eichhorn, et al. 2017. "Different Potential of Extracellular Vesicles to Support Thrombin Generation: Contributions of Phosphatidylserine, Tissue Factor, and Cellular Origin." *Scientific Reports* 7: 6522.

Tsuchiya, A., S. Terai, I. Horiguchi, et al. 2022. "Basic Points to Consider Regarding the Preparation of Extracellular Vesicles and Their Clinical Applications in Japan." *Regenerative Therapy* 21: 19–24.

Turchiano, G., G. Andrieux, J. Klermund, et al. 2021. "Quantitative Evaluation of Chromosomal Rearrangements in Gene-Edited Human Stem Cells by CAST-Seq." *Cell Stem Cell* 28: 1136–1147.e5.

Vagner, T., A. Chin, J. Mariscal, S. Bannykh, D. M. Engman, and D. Di Vizio. 2019. "Protein Composition Reflects Extracellular Vesicle Heterogeneity." *Proteomics* 19: e1800167.

van der Pol, E., F. A. W. Coumans, A. E. Grootemaat, et al. 2014. "Particle Size Distribution of Exosomes and Microvesicles Determined by Transmission Electron Microscopy, Flow Cytometry, Nanoparticle Tracking Analysis, and Resistive Pulse Sensing." *Journal of Thrombosis and Haemostasis* 12: 1182–1192.

van de Wakker, S. I., J. Bauzá-Martinez, C. Ríos Arceo, et al. 2024. "Size Matters: Functional Differences of Small Extracellular Vesicle Subpopulations in Cardiac Repair Responses." *Journal of Extracellular Vesicles* 13: e12396.

van Niel, G., D. R. F. Carter, A. Clayton, et al. 2022. "Challenges and Directions in Studying Cell-Cell Communication by Extracellular Vesicles." *Nature Reviews Molecular Cell Biology* 23: 369–382.

Vaz, I. M., T. Borgonovo, T. H. Kasai-Brunswick, et al. 2021. "Chromosomal Aberrations After Induced Pluripotent Stem Cells Reprogramming." *Genetics and Molecular Biology* 44: e20200147.

Verweij, F. J., L. Balaj, C. M. Boulanger, et al. 2021. "The Power of Imaging to Understand Extracellular Vesicle Biology In Vivo." *Nature Methods* 18: 1013–1026.

Vestad, B., A. Llorente, A. Neurauter, et al. 2017. "Size and Concentration Analyses of Extracellular Vesicles by Nanoparticle Tracking Analysis: A Variation Study." *Journal of Extracellular Vesicles* 6: 1344087.

Vogel, R., F. A. W. Coumans, R. G. Maltesen, et al. 2016. "A Standardized Method to Determine the Concentration of Extracellular Vesicles Using Tunable Resistive Pulse Sensing." *Journal of Extracellular Vesicles* 5: 31242.

Walker, S. A., I. Davidovich, Y. Yang, et al. 2022. "Sucrose-Based Cryoprotective Storage of Extracellular Vesicles." *Extracellular Vesicle* 1: 100016.

Wang, C. K., T. H. Tsai, and C. H. Lee. 2024. "Regulation of Exosomes as Biologic Medicines: Regulatory Challenges Faced in Exosome Development and Manufacturing Processes." *Clinical and Translational Science* 17: e13904.

Wang, J., L. Liu, X. Gao, et al. 2025. "A Novel Pathway for Stemness Propagation and Chemoresistance in Non-Small Cell Lung Cancer via Phosphorylated PKM2-Loaded Small Extracellular Vesicles." *Theranostics* 15: 3439–3461.

Wang, Y., Q. Yang, D. Fang, L. Sun, L. Zheng, and D. Dou. 2023+. "Progress in Exosome-Related Research Supported by the National Natural Science Foundation of China." *Fundamental Research* 5: 2318–2329.

Wang, Y.-L., C. C.-Y. Huang, C.-M. Zheng, W.-C. Liu, Y.-H. Lee, and H.-W. Chiu. 2024. "Polystyrene Microplastic-Induced Extracellular Vesicles Cause Kidney-Related Effects in the Crosstalk Between Tubular Cells and Fibroblasts." *Ecotoxicology and Environmental Safety* 273: 116098.

Wang, Z. 2023. "Assessing Tumorigenicity in Stem Cell-Derived Therapeutic Products: A Critical Step in Safeguarding Regenerative Medicine." *Bioengineering* 10: 857.

Wang, Z., X. Zhou, Q. Kong, et al. 2024. "Extracellular Vesicle Preparation and Analysis: A State-of-the-Art Review." *Advanced Science* 11: e2401069.

Webber, J., and A. Clayton. 2013. "How Pure Are Your Vesicles?" *Journal of Extracellular Vesicles* 2: 19861.

Weber, B., W. Welz, I. Schaible, et al. 2025. "Influence of Hemolysis, Lipemia and Bilirubin on Biobank Sample Quality-Origin and Interference in the Use for Extracellular Vesicle (EV) and MiRNA Analyses." *European Journal of Trauma and Emergency Surgery* 51: 153.

Welsh, J. A., D. C. I. Goberdhan, L. O'Driscoll, et al. 2024. "Minimal Information for Studies of Extracellular Vesicles (MISEV2023): From Basic to Advanced Approaches." *Journal of Extracellular Vesicles* 13: e12404.

Welsh, J. A., E. van der Pol, B. A. Bettin, et al. 2020. "Towards Defining Reference Materials for Measuring Extracellular Vesicle Refractive Index, Epitope Abundance, Size and Concentration." *Journal of Extracellular Vesicles* 9: 1816641.

Williams, B., R. Kozar, and W. Chao. 2023. "Emerging Role of Extracellular RNA in Innate Immunity, Sepsis, and Trauma." *Shock* 59: 190–199.

Williams, S., M. Fernandez-Rhodes, A. Law, B. Peacock, M. P. Lewis, and O. G. Davies. 2023. "Comparison of Extracellular Vesicle Isolation Processes for Therapeutic Applications." *Journal of Tissue Engineering* 14: 20417314231174609.

Wolf, M., R. W. Poupardin, P. Ebner-Peking, et al. 2022. "A Functional Corona Around Extracellular Vesicles Enhances Angiogenesis, Skin Regeneration and Immunomodulation." *Journal of Extracellular Vesicles* 11: e12207.

Woo, J., S. Sharma, and J. Gimzewski. 2016. "The Role of Isolation Methods on a Nanoscale Surface Structure and Its Effect on the Size of Exosomes." *Journal of Circulating Biomarkers* 5: 11.

Wu, Y., W. Deng, and D. J. Klinke 2nd. 2015. "Exosomes: Improved Methods to Characterize Their Morphology, RNA Content, and Surface Protein Biomarkers." *Analyst* 140: 6631–6642.

Xu, G., J. Jin, Z. Fu, et al. 2025. "Extracellular Vesicle-Based Drug Overview: Research Landscape, Quality Control and Nonclinical Evaluation Strategies." *Signal Transduction and Targeted Therapy* 10: 255.

Yang, C., J. Han, H. Liu, et al. 2024. "Storage of Plasma-Derived Exosomes: Evaluation of Anticoagulant Use and Preserving Temperatures." *Platelets* 35: 2337255.

Yang, L., J. Li, S. Li, et al. 2021. "Extracellular Vesicles Regulated by Viruses and Antiviral Strategies." *Frontiers in Cell and Developmental Biology* 9: 722020.

Yang, Y.-H. K., C. R. Ogando, C. Wang See, T.-Y. Chang, and G. A. Barabino. 2018. "Changes in Phenotype and Differentiation Potential of Human Mesenchymal Stem Cells Aging In Vitro." *Stem Cell Research & Therapy* 9: 131.

Yates, A. G., R. C. Pink, U. Erdbrügger, et al. 2022. "In Sickness and in Health: The Functional Role of Extracellular Vesicles in Physiology and Pathology In Vivo: Part I: Health and Normal Physiology: Part I: Health and Normal Physiology." *Journal of Extracellular Vesicles* 11: e12151.

Yousefian, F., L. Espinoza, S. Yadlapati, Z. P. Lorenc, and M. Gold. 2024. "A Comprehensive Review of the Medical and Cosmetic Applications of Exosomes in Dermatology." *Journal of Cosmetic Dermatology* 23: 1224–1228.

Zaborowski, M. P., L. Balaj, X. O. Breakefield, and C. P. Lai. 2015. "Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study." *Bioscience* 65: 783–797.

Zhang, H., D. Freitas, H. S. Kim, et al. 2018. "Identification of Distinct Nanoparticles and Subsets of Extracellular Vesicles by Asymmetric Flow Field-Flow Fractionation." *Nature Cell Biology* 20: 332–343.

Zhang, R., X. Qin, F. Kong, P. Chen, and G. Pan. 2019. "Improving Cellular Uptake of Therapeutic Entities Through Interaction With Components of Cell Membrane." *Drug Delivery* 26: 328–342.

Zhang, W., B. Gan, T. Wang, et al. 2023. "Extracellular Vesicles in the Treatment of Oxidative Stress Injury: Global Research Status and Trends." *Frontiers in Molecular Biosciences* 10: 1273113.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Zhou, X., J. Huang, D. Zhang, Z. Qian, X. Zuo, and Y. Sun. 2025. “Small Extracellular Vesicles: The Origins, Current Status, Future Prospects, and Applications.” *Stem Cell Research & Therapy* 16: 184.

20013078, 2026, 5, Downloaded from https://isevjournals.onlinelibrary.wiley.com/doi/10.1002/jev2.70297, Wiley Online Library on [11/05/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License