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Exploring Regulatory Frameworks for Exosome Therapy: Insights and Perspectives

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ABSTRACT

Extracellular vesicles (EVs) have emerged as a promising technology for diagnostic and therapeutic applications in clinical settings over the past decade. However, their advancement is hindered by complex technological and regulatory challenges. This review outlines key considerations in the manufacturing process, quality management, and nonclinical evaluation relevant to EV-based drug development. Furthermore, we summarize and compare technical regulatory requirements across major countries to help clarify the regulatory principles governing EV products. Our analysis reveals an ongoing international debate regarding the regulatory review of EVs. Nevertheless, adopting a risk-based classification framework that categorizes EV products as advanced therapeutic drugs is a rational approach. Critical challenges include the development of standardized production protocols, a clearer understanding of therapeutic mechanisms, and resolving complex regulatory issues.

1 | Introduction

Extracellular vesicles (EVs) are a diverse group of membrane-bound structures derived from the endosome or plasma membrane. Initially, their release serves as a cellular mechanism for eliminating unwanted materials. Subsequent research has shown that EVs constitute a component of the cellular secretome, which is involved in intercellular communication. EVs cannot replicate [1, 2] and are subcellular structures enclosed by a lipid bilayer that resembles the plasma membrane.

2 | Biogenesis of EVs

All cell types can secrete EVs, which originate from the endocytic compartment of the producing cell and are subsequently secreted into bodily fluids. Thus, EVs can be collected and purified from bodily fluids or the extracellular environment. EVs contain biomolecules from the producer (parent) cell that are released either spontaneously or after induction [3]. The generic name "EV" encompasses particles of various types secreted by cells, including EVs, microvesicles (MVs)/ectosomes, microparticles, apoptotic bodies (ABs), and small,

Abbreviations: EMEA, European Medicines Agency; EV, extracellular vesicle; FDA, food and drug administration; GMP, good manufacturing practice; MFDS, Ministry of Food and Drug Safety; MOA, mode of action; PMDA, Pharmaceuticals and Medical Devices Agency.

medium, and large vesicles [4]. The fusion of various EVs produces endosomes that develop into multivesicular bodies (MVBs) [5]. Two principal sources of synthesized MVB have been identified: the formation of intermediates involved in intracellular protein degradation or EV synthesis [6].

EVs, their biogenesis, final products, and characters such as shape and size show distinct differences from MVs and apoptotic bodies, which are larger, morphologically diverse, and surface-marker confined (Figure 1).

In contrast to the generation of other MVBs, which is primarily driven by the budding of the cell membrane, the biosynthesis of EVs is characterized by a greater degree of complexity. Originating from endosomes, EVs are distinctly characterized by the presence of endosomal components, including specific biomarkers like CD63, CD9, and CD81, and a variety of proteins, such as transcription factors and oncogenic regulators [7]. The protein composition of EVs serves as a reliable indicator of their subtype, providing insights into their biogenesis, release mechanisms, and cellular origin. Specific proteins are typically present in EVs, regardless of their source, including heat shock proteins (HSP84, HSC70, and HSP90β), tumor susceptibility gene 101 (TSG101), and Alix. EVs carry genetic material from their parental cells, such as microRNAs, long noncoding RNAs, and circular RNAs [8].

2.1 | Key Characteristics

EVs consist of a lipid bilayer abundant in unsaturated fatty acids, phosphatidylserine, polyglycerol, sphingomyelin, cholesterol, and gangliosides. The density of EVs typically falls within the range of 1.08 to 1.22 g/mL [9]. The lipid bilayer of EVs provides structural strength, stiffness, and a rigid environment that serves as a barrier to prevent enzymatic degradation, allowing for greater stability as a carrier molecule. Newly synthesized EVs contain more lipids, including cholesterol, phosphatidylserine, and sphingomyelin, and less lysophosphatidic (bis)phosphatidic acid and phosphatidylcholine [10].

2.2 | Biological Features

The primary function of EVs is to facilitate the transfer of biomolecular cargo from host cells to recipient cells, thereby mediating intercellular communication. Since their discovery, EVs have been demonstrated to play a pivotal role in maintaining normal physiology and in mediating pathological conditions. EVs are gaining considerable interest because of their role in cell biology and potential applications in therapy and diagnostics. This cell-to-cell communication supports a wide range of biological functions in both healthy and diseased states and offers the opportunity to develop new drug delivery systems.

Numerous clinical benefits of EVs have been described, rendering these vesicles promising candidates for therapeutic delivery, disease progression inhibition, and biomarkers for disease monitoring. Nevertheless, a more comprehensive understanding of the precise molecular pathways and biogenesis that give rise to the various subtypes of EVs is required to maximize the potential of EVs in clinical applications.

3 | Naïve EV and Engineered EVs: Clinical Potential

EVs can undergo engineering either before or after production to incorporate native or synthetic molecules, thereby enhancing their specific targeting or therapeutic properties. Therefore, EVs can be classified into two major categories: naïve EVs and engineered EVs [11, 12].

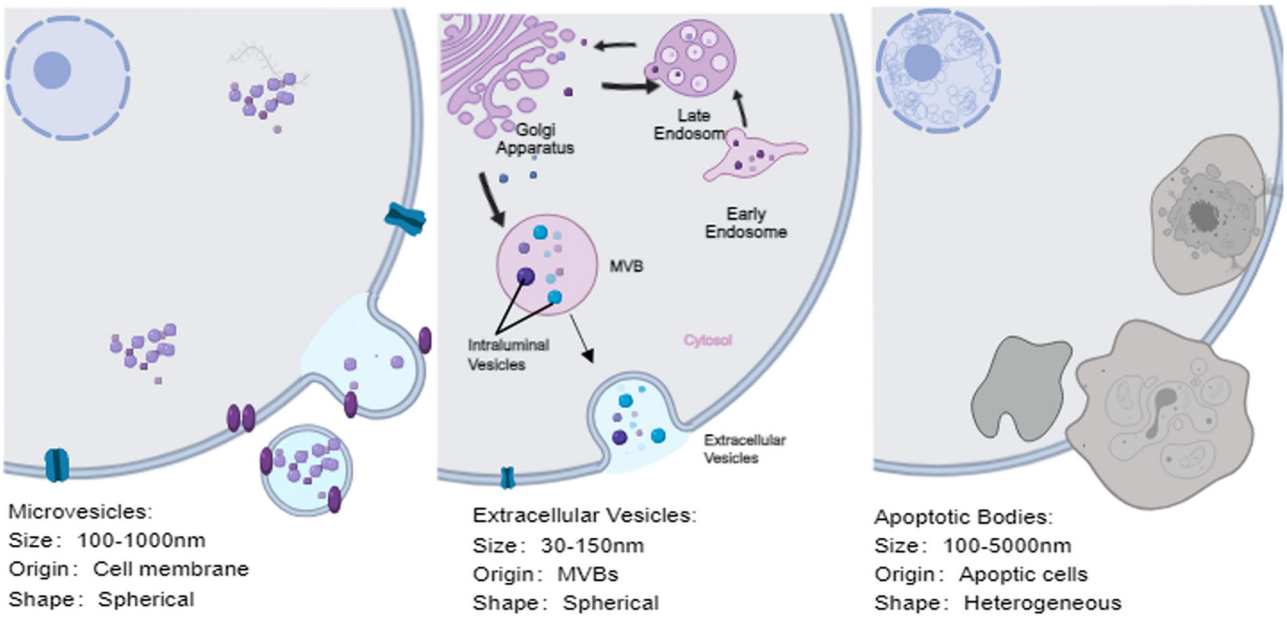

FIGURE 1 | Biogenesis of microvesicles, extracellular vesicles, and apoptotic bodies.

3.1 | Naïve EV

The EVs of mesenchymal stem cells (MSCs) are the most concentrated native source under research. MSCs exhibit a wide range of therapeutic efficacy in various preclinical models of immunological and degenerative diseases, with a proven history of safety in human patients [13]. Although MSCs were initially referred to as "mesenchymal stem cells" and believed to act therapeutically as stem cells via cellular differentiation and cell replacement, it is now clear that the effects of MSCs are primarily mediated by paracrine factors, with a significant proportion of these effects being exerted through the release of EVs. MSC-EVs are essentially miniature versions of their parental cells, in part because they are derived from a specific cell type and provide a unique set of biomolecules. The application of MSCs as a form of cell therapy is based on their ability to regulate the inflammatory response and participate in tissue repair and regeneration processes. In response to inflammatory stimuli, MSCs secrete a multitude of immunomodulatory factors, chemokines, and growth factors that regulate the tissue immune microenvironment and facilitate tissue regeneration. Currently, almost 1000 clinical trials have been registered to assess the administration of MSCs derived from a variety of sources, including bone marrow (BM), adipose tissue (AD), and cord blood (CB) (e.g., NCT03484741 for type 1 diabetes mellitus, NCT02687646 for graft vs. host disease, and NCT03635450 for hypoxic-ischemic encephalopathy, respectively). The majority of these trials are focused on using MSCs in hematopoietic stem cell transplantation (HSCT) (NCT05290545), tissue healing (NCT01733186 for articular cartilage), autoimmune diseases (AID) (NCT06737380 for lupus, NCT03828344 for rheumatoid arthritis, as examples), and genetic therapy vectors.

Various pharmaceutical-grade, naïve extracellular vesicles are in early-stage clinical development. Several companies focused on the production and commercialization of exosomes have begun clinical translation of extracellular vesicles as therapeutics, with Direct Biologics (NCT04493242 and NCT05354141 for respiratory failure from COVID19 treatment [14]), Aegle Therapeutics (NCT04173650 for dystrophic epidermolysis bullosa), and Rion (NCT06319287 for diabetic foot ulcer) representing leading players with several active phase I/II clinical trials. Kimera Labs produced one of the first exosomes to achieve pharmaceutical-grade status by meeting the FDA requirements for treating ARDS secondary to COVID-19.

3.2 | Engineered EVs

Beyond natural processes, a range of engineered methods can be employed to introduce specific cargo into EVs. These approaches are categorized into two main strategies based on the timing of content addition: endogenous and exogenous loading [15]. Endogenous loading occurs at the cellular level, utilizing parent cells to incorporate therapeutic molecules into EVs during biogenesis. Techniques such as transfection and co-incubation are used to modify parent cells, enabling the packaging of desired cargoes into EVs as they form. However, this method often affords limited yields and variable loading efficiencies, posing challenges for large-scale applications.

EVs are envisioned as excellent delivery platforms in biomedicine because of their low toxicity, minimal risk of an immune response, long in vivo circulation, nanoscale size for deep tissue penetration, multi-cargo loading capability, and surface molecular editing potential. Specifically, EVs can be packaged through co-incubation, enabling the diffusion of cargo across both cellular and EV membranes. Alternatively, desired nucleic acids can be loaded into EVs via transfection-based strategies. Cargoes can also be loaded directly into EVs by physical treatment. Electroporation, sonication, and surfactant treatment create pores in the EV membrane to facilitate cargo loading. Finally, in situ assembly and synthesis facilitate the loading of metal nanoparticles by reducing metal ions to nanoparticles within the EV. The above methods facilitate the production of EVs with a large number of target molecules that are protected from in vivo environmental damage, making EVs a desirable mode of drug delivery.

In contrast, exogenous loading involves directly introducing cargo into pre-isolated EVs through methods such as co-incubation, ultrasonic treatment, electroporation, and specialized EV transfection kits [16, 17]. Exogenous techniques offer greater convenience and control over cargo loading, making these approaches popular in developing novel delivery systems. Notably, transfection kits have gained widespread use because of their simplicity, speed, and high loading efficiency. These engineered EVs have been shown to effectively deliver miR-155-5p or other therapeutic agents to target cells, facilitating intracellular trafficking and achieving the desired therapeutic outcomes [18].

Despite the wide variety of engineering methods, which are diverse and show therapeutic advantages, the process reliability of EV engineering is poorer than that of other artificially engineered nanovesicles. Firstly, the yield of EVs is severely limited by the ability of cells to secrete various EVs, the high difficulty and cost of large-scale cell culturing, and the time-consuming and inefficient methods of EV isolation and purification. These drawbacks significantly hamper the industrial production of EVs [19].

Secondly, EVs have limited cargoloading efficiency. EVs are inherently packaged with natural proteins and nucleic acids that significantly increase the difficulty of loading the required cargo [20]. Although several methods for designing EVs to increase loading capacity have been discussed previously, the cargo loading efficiency of EVs remains significantly lower than that of unpackaged synthetic liposomes [21].

Thirdly, quality control of EVs is more challenging than that of other approaches. EVs, even when produced by a single cell type, are highly heterogeneous. The lack of highly sensitive, high-throughput analysis of low-copy-number nucleic acids and proteins in the single EV dimension hinders the separation of heterogeneous EV populations, thereby yielding heterogeneous samples [22]. In addition, EVs may inherit unwanted macromolecules from their parental cells because an important function of EVs is to remove harmful or unwanted substances from cells [23, 24].

Harnessing EVs as natural carriers for delivering drugs or nucleic acids to target cells represents a promising strategy for

developing therapeutic approaches for various diseases. Their biocompatibility, ability to cross biological barriers, and potential for targeted delivery make EVs an innovative platform for advanced drug delivery systems [25, 26].

3.3 | Clinical and Therapeutic Advantages

EVs hold significant potential in various medical applications, including serving as biomarkers for disease diagnosis, acting as drug delivery vehicles or therapeutic agents, and functioning as immunomodulators to either stimulate or suppress the immune response. The capacity to isolate EVs from MSC cultures as a cell-free therapy that can circumvent immune rejection and tumorigenesis following injection represents a significant advantage and a comparatively safer treatment option [27].

EVs are distinguished by their lack of immune rejection and malignancy risk, stability, long-term maintenance, and ability to cross biological barriers. These attributes open up novel therapeutic strategies for treating various diseases [28–30]. Notably, drug delivery across the blood–brain barrier (BBB) poses a significant challenge because the barrier is impermeable to most therapeutic agents [31–33]. However, studies have shown that natural and bioengineered EVs loaded with drugs can successfully penetrate the BBB and remain stable in peripheral circulation [34, 35]. Additionally, EVs can cross the maternal–placental barrier [36, 37], further highlighting their potential as a versatile therapeutic platform for various applications [38].

4 | EV: Technological Management and Common Questions

4.1 | Purification, Characterization, and Control

The primary challenge in preparing EVs to carry various cargoes lies in upscaling cell cultures while maintaining the stability of cellular features. The expansion of stem cells is hindered by their inherent potential to differentiate into a variety of cell types, which may result in the release of EV mixtures with unpredictable properties [39]. Scaling EV purification represents the next bottleneck because the small, low-density, and widely distributed EVs must be obtained from complex fluid environments. Thus, isolation is a critical stage in the manufacturing process, as it directly influences the purity, yield, and overall cost of EV-based therapeutics. Several new techniques and commercial products have been developed for isolating EVs [40–42].

Some of these techniques take full advantage of the general physicochemical properties of EVs, such as density, mass, and shape, to separate EVs from body fluid. EVs can also be enriched and separated based on other physicochemical and biochemical properties, such as charge, hydrodynamics, solubility, and surface properties [43, 44]. Isolation techniques for EVs include differential centrifugation (DC), precipitation, size exclusion chromatography (SEC), ultrafiltration (UF), tangential flow filtration (TFF), and immunocapture. Each method has distinct advantages and drawbacks. Currently, however, there is no single gold standard method for isolating EVs [45]. DC is widely regarded as a standard technique for obtaining pure EVs; however, this approach is time-consuming and costly. In contrast, precipitation offers a quicker and more straightforward approach, suitable for large-scale production, albeit with reduced purity. TFF is a highly efficient and high-resolution separation technique for obtaining EV samples with high purity, meeting the requirements of industrial-scale manufacturing [46]. Nonetheless, achieving high EV purity may not be essential in specific applications. For example, the full MSC-secretome, which includes soluble proteins and EVs, has demonstrated therapeutic efficacy. Therefore, an emphasis on ultrapure EVs may not always be warranted. The manufacture of EV-based products has long been recognized as necessitating the implementation of a quality management system that accounts for the safety of both donors and recipients [47]. Consequently, when developing EV-based therapeutics, the choice and characterization of the EV source for cGMP production are of utmost relevance [48]. In this context, characterizing EV formulations, identifying EV markers, and determining purity and quantity represent additional challenges that are equally important aspects of quality control measures and process parameter definitions [49, 50].

Challenges include insufficient EV secretion by cells for clinical translation, low yield per mL of culture medium, time-consuming or expensive isolation methods, a lack of clinically feasible methods for large-scale EV production, and the absence of good manufacturing practices (GMPs) that comply with production protocols to ensure EV quality. For example, while the dose used in most studies is approximately 10–100 μg EV protein/mouse, the yield of EVs per 1 mL of culture medium is typically less than 1 μg EV protein/mouse.

4.2 | Process, Formulation, and Storage

Prior to their clinical applications, the production, purification, and modification of EVs must be optimized, and preclinical pharmacotoxicological and pharmacokinetic studies are also required during the preclinical development phase. Under established norms of the pharmaceutical industry, the chemistry, manufacturing, controls (CMC), and both clinical and nonclinical requirements are meticulously considered well in advance of phase I clinical trials. This approach ensures that a cGMP-compliant product is ultimately available for clinical use.

Storage protocols for EV-based therapeutics must consider factors such as temperature, buffer composition, and storage containers to ensure prolonged shelf life. Siliconized vials are recommended to minimize EV adhesion and loss during purification and storage [51]. Phosphate-buffered saline (PBS) is typically used as a storage medium. The most widely accepted storage method is freezing at −80°C, as this preserves the EV characteristics. In contrast, storage at 4°C leads to EV damage and aggregation. However, maintaining ultra-low temperatures poses challenges for transport and increases costs. Interestingly, freeze-drying (lyophilization) has emerged as a promising alternative. According to Frank et al. [52], lyophilization does not significantly affect the size and number of particles in MSC-derived EVs. Additionally, the inclusion of cryoprotecting sugars helps maintain the activity of enzymes in the lyophilized

EVs, with activity comparable to enzymes stored at −80°C. This method may offer a cost-effective and logistically feasible solution for EV storage and transport while preserving therapeutic efficacy.

4.3 | Nonclinical Pharmacology and Toxicology Studies

EVs, as promising therapeutic tools, possess native structures and characteristics that make them ideal nanocarriers. These features include small molecular sizes to facilitate deep tissue penetration [53], slightly negative zeta potentials to ensure prolonged circulation [54], a deformable cytoskeleton [55], and resemblance to cell membranes. Moreover, EVs can express specific surface proteins and carry therapeutic agents, allowing targeted delivery to affected tissues while minimizing off-target effects. Their structure enables the encapsulation of hydrophilic compounds in the core and hydrophobic molecules in the lipid bilayer. However, comprehensive research is required to characterize the molecular cargo of EVs, assess their pharmacokinetic profiles, and investigate their therapeutic effects across various disease models.

For nonclinical development, a particularly challenging hurdle is the selection of a representative animal model, which should be identified and established well in advance of clinical studies investigating EV-based therapeutics. As with all cell-derived therapeutics, this model must enable the assessment of safety, toxicity, biodistribution, pharmacokinetics/pharmacodynamics profile, immunogenicity, and tumorigenicity [56, 57]. A risk-based approach, as laid out for advanced therapy medicinal products (ATMPs) by the EMA, should be considered where necessary. Early-stage in vitro biological characterization and potency assessment should be conducted in conjunction with nonclinical in vivo studies.

5 | Regulatory Aspects

5.1 | Regulatory Pathway: MedTech, Device, and Drug

As mentioned, EVs as therapeutic drugs differ from traditional drugs in terms of composition, active substances, production, and preparation. Additionally, the regulation, review, and release according to EV quality, activity, and safety remain open questions. Based on experience from global authorities, EVs are not treated as a separate review classification but are placed within the general biological drug review framework, according to their therapeutic properties, or along existing fast-track review pathways, depending on their therapeutic potential [58].

From an operational viewpoint, EV products can be classified into various categories, including medical technology and cosmetics. Under earlier product registration frameworks, particularly in China, stem cell-derived EVs were classified as medical technologies. Hospitals, as the designated implementers of such technologies, assumed responsibility for ensuring the quality, clinical safety, and efficacy of EV use. However, medical technology is often confined to hospitals, which hinders its industrialization and management if the technology developer is not located within a hospital.

EV products are currently applied in cosmetic form worldwide. Despite the widespread benefits of EVs in skin rejuvenation, skin wound healing, atopic dermatitis, melasma, and skin manifestations of systemic diseases, no clear regulatory guidelines have been issued in major countries. The US Food and Drug Administration (USFDA) has not formally categorized EVs as cosmetics; therefore, it is unclear what type of regulation should be implemented. In the European Union, EVs used in cosmetics may be regulated by the European Commission, which may include restrictions on manufacturing, labeling, and advertising. In Japan and South Korea, despite the growing marketing of EVs, the regulation of EV-based cosmetics remains largely absent.

In terms of product quality and safety, EV products should be approved as biological products or drugs. Biological products refer to preparations made from microorganisms, cells, tissues, and body fluids of animal or human origin as starting raw materials and are produced using biological technology for the prevention, treatment, and diagnosis of human diseases. To standardize the registration, declaration, and management of biological products, these products are divided into three categories: biological products for prevention, biological products for treatment, and in vitro diagnostic reagents, which are managed separately as biological products.

The regulatory environment for EV therapies is complex and varies from country to country. In terms of EV composition, the diversity of cell sources, culture conditions, purification methods, and manufacturing technologies makes their standardization challenging. Moreover, industries in each country are shaped by different technological routes, and the lack of commonality in the problems encountered in regulation has created distinct problems for the review bodies in each country, leading to a fragmented regulatory environment.

The development of EV-based therapeutics must align with current regulatory frameworks to ensure compliance with established standards for safety, efficacy, quality, and consistency. Developers are required to adhere to GMP and fulfill regulatory obligations under investigational new drug (IND) guidelines. This adherence necessitates rigorous preclinical and clinical testing protocols that demonstrate the therapeutic safety and effectiveness of EV products. Regulatory approval depends on comprehensive data showcasing the reproducibility and stability of the final product.

Regulatory adaptation to EV characteristics is essential to address the unique properties of EVs, such as their inherent heterogeneity and diverse mechanisms of action. To support the clinical translation of EV-based therapeutics, regulatory frameworks may need to evolve by developing specific criteria for EV characterization, implementing stringent quality control measures, and designing potency assays and stability testing protocols that account for the biological complexity of EVs. Establishing clearer approval pathways that reflect these unique attributes would also facilitate product development. Ongoing dialogs and collaborations among regulatory bodies, academic

researchers, and industry stakeholders will be crucial in refining guidelines that meet the distinct challenges associated with EV products. Addressing questions that facilitate the safe and effective development of EV-based therapies is also required.

As the therapeutic applications of EV therapy expand, the International Society for Extracellular Vesicles (ISEV) and the European Network on Microvesicles and EVs in Health and Disease (ME-HaD) have been established to provide baseline criteria for the clinical application of EVs. These baseline requirements should serve as the starting point for quality control and facilitate the development of additional criteria for evaluating and approving products. By adhering to these standards, the therapeutic potential of EVs can be harnessed safely and effectively, ensuring compliance with appropriate quality benchmarks for clinical applications.

5.2 | Regulatory Principles From USFDA, European Medicines Agency (EMEA), Pharmaceuticals and Medical Devices Agency (PMDA), and Ministry of Food and Drug Safety (MFDS)

The development of any therapy or drug relies heavily on standardized processes to validate the proposed technology. However, the lack of regulation has led to a rise in direct-to-consumer businesses offering stem cell "therapies" for various indications, often with minimal evidence supporting their safety and efficacy [59]. Currently, no EV products have received approval from the FDA for human use. The FDA classifies EVs as a 351 products, meaning they require extensive studies demonstrating safety, efficacy, purity, and potency before approval. EV-based therapies are currently in the IND phase, requiring regulatory agency approval before clinical trials can commence [60].

The current global regulatory framework for EVs can be broadly categorized into two main groups, depending on how the regulator defines the class of products: one approach can be viewed as composition-based, that is, centered on elucidating the impact of EV content on physiological function, as favored by drug regulatory authorities in the US and Europe. This understanding implies that the need to define the active basis of EVs is at the center of product regulation, with the method of preparation being placed in second place. The second scenario, represented by countries such as Japan and South Korea, is that the method of obtaining EVs and their source becomes central to defining EV drugs, a regulatory framework that implies that similar cells become the same drug via the same method of preparation, and that the function of EVs derives from its function in the parent cell. However, this situation also raises regulatory concerns, as product validation remains a challenge in addition to noncellular characterization and purification selectivity.

Clearly, the drug classification of EV-based therapies must depend on the active substance these vesicles carry. The regulatory classification of any drug and most biopharmaceutical products depends on the drug's active substance, which does not necessarily have to be a defined molecule and, in the case of cell-based therapies, can be the cell. Manufacturers are required to identify, quantify, and characterize the primary substance in a drug that causes a particular pharmacological, immunological, or metabolic effect and are required to ensure that these effects are linked to the biological effects of the drug (i.e., the mode or mechanism of action [MoA]). Inactive ingredients (excipients) required in the final formulation of the drug must also be characterized.

The MoA of EV-based therapeutics, which can be the vesicle contents, the membrane, or a combination of both, must be addressed during development. For many therapeutic applications, the precise MoA may be undefinable, even if the treatment is effective. Ideally, the biological activity of EVs should be established using a relevant, reliable, and qualified method before phase I clinical trials [61, 62]. Additionally, the rationale for selecting characterization methods should be provided, and their appropriateness must be validated. Although the specifics are to be determined on a case-by-case basis with regulators, the ISEV proposes a framework for categorizing EV-based therapeutics based on the nature of their active substances.

In the first scenario, native EVs derived from unmodified cells and containing only native components are categorized as biological medicines. Thus, the EVs function as active substances through their overall composition, enabling them to enter recipient cells and influence downstream pathways.

The second scenario involves EVs from genetically modified cells without transgene products. These EVs are similarly classified as biological medicines, with therapeutic effects attributed to the combined action of their membrane composition and cargo molecules. Distinguishing between the contributions of individual components may not be necessary.

In the third scenario, EVs containing transgene products released from genetically modified cells are classified as gene therapy products (GTPs) under ATMPs. This classification depends on whether the therapeutic effect is primarily because of the transgene product or the EVs.

Finally, native EVs used as drug-delivery systems for chemical drugs or molecular components, such as miRNAs or siRNAs, are categorized as either combined biological and chemical therapeutics or biological medicines. Determining whether the EVs contribute to the therapeutic effect is crucial in assessing their role as part of the active substance.

This categorization framework underscores the importance of rigorous characterization and clear definitions to ensure compliance with regulatory standards (Figure 2).

5.2.1 | USFDA

In the US, the trend of expediting the investigation of stem cell products in human patients is also reflected in the study of EV-based therapeutics. EVs, as novel and therapeutically active substances intended for clinical use, are classified as INDs. Advancing an IND to clinical trials requires submitting an IND application following preclinical development. This application must include information about prior animal studies,

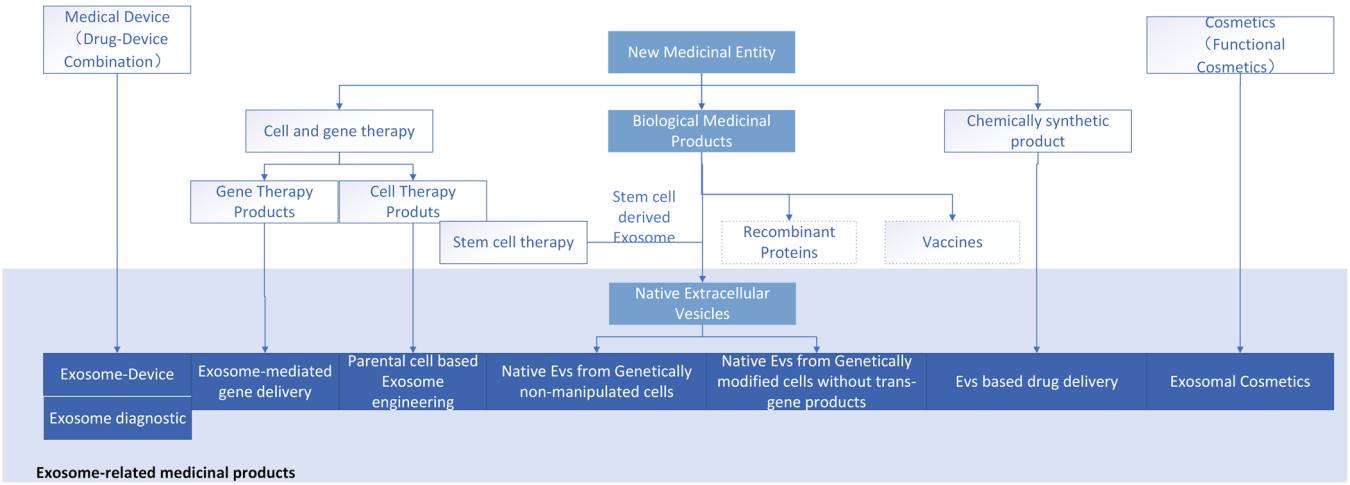

FIGURE 2 | The filing pathway for EVs. EVs as drug-device combinations, cosmetic products, and new medicinal entities. For historical reasons, stem cells have represented the primary source of therapeutic EVs, with the two most central classifications being native EVs produced from genetically non-manipulated cells and EVs that have been genetically engineered with cells but without transgene products. EVs have also been tried as carriers in gene delivery and as small-molecule delivery systems. Concurrently, more diversified applications, such as pharmaceutical devices and cosmetics, are posing more complex challenges for reviewing EVs carrying drug cargo.

manufacturing information, clinical protocols, and investigator details. Furthermore, EVs, defined as "a medicine that contains one or more active substances made by or derived from a biological cell," are categorized as biological medicinal products and are regulated by the FDA's Center for Biologics Evaluation and Research (CBER). For an IND application, investigators must detail technical specifications critical to the clinical feasibility and outcomes of EV-based therapeutics. These include identifying optimal EV sources (e.g., donors, cells, tissues, fluids) and describing strategies for EV production, isolation, purification, characterization, and storage. Establishing rigorous quality control is essential for ensuring that the biological, molecular, and physical properties of EVs remain consistent across production batches, thus ensuring uniform therapeutic effects. However, because of the inherent heterogeneity of EVs, standardizing purification processes and release criteria presents challenges. Investigators must demonstrate to the FDA that this heterogeneity is a natural biological trait with potential therapeutic benefits rather than a feature that requires resolution. Additionally, investigators are required to provide comprehensive in vitro and in vivo data that demonstrate the pharmacological and toxicological profiles of EVs, ensuring their safety and efficacy as therapeutic agents. Clinical protocols must also be established, specifying the route of administration, dosage, and frequency of treatments as part of the overall preclinical and clinical development process.

5.2.2 | EMEA

The European Union has categorized cell and gene therapy products as ATMPs, and the overall framework for ATMPs is provided by Regulation (EC) No 1394/2007. Moreover, the EU has established the Committee for Advanced Therapies (CAT), which comprises experts from various fields, to review the quality, safety, and efficacy of ATMPs and to enhance communication with companies. The EMA has also issued several guidelines, opinions, action plans, and, specifically for ATMPs, to promote their efficient development. ATMP applies to a centralized review process that is based on establishing a precategorization process.

ATMPs are also subject to various accelerated approval routes offered by the EMA, such as Priority Medicines (PRIME), conditional approval, and marketing authorization under exceptional circumstances. Additionally, many ATMPs are not suitable for healthy volunteer studies for ethical reasons. Thus, initial time-in-human (FTIH) trials enroll patients in combined Phase I/II trials to assess safety and initial efficacy. Efficacy is then confirmed in a subsequent Phase III or pivotal clinical trial, which is used to support EMA marketing approval.

5.2.3 | PMDA

In Japan, under the Pharmaceuticals and Medical Devices Act, which regulates the manufacture and distribution of therapeutic products intended for marketing, cell and gene therapy products are categorized under a distinct classification known as "regenerative medical products," and a unique conditional and time-limited approval system may apply to regenerative medical products [63]. EVs that do not contain living cell components are not classified as regenerative medical products or medical devices but are typically categorized as drugs, acting primarily through pharmacological, immunological, or metabolic mechanisms [64]. Furthermore, noncommercial clinical research on EVs and medical treatments involving EVs conducted independently by medical practitioners fall outside the scope of the Act on the Safety of Regenerative Medicine. This exemption applies to EVs that do not contain viable cell components and are not considered specified processed cells.

The applicant proposes to the PMDA, which reviews and makes recommendations, submitting them to the Ministry of Health, Labour and Welfare (MHLW). The MHLW then consults with the Pharmaceutical and Food Hygiene Board based on the

results of the review and makes the final decision. Equivalent to a conditional time-limited approval, the maximum duration of the conditional/time-limited license is 7 years. After demonstrating the clinical effectiveness of the cell therapy product, the lead researcher can reapply for long-term marketing as a full regenerative medicinal product. This pathway applies primarily to products that are difficult to demonstrate efficacy in a short period and may require conditional approval if it can be demonstrated in the early stages of a clinical trial that the product is likely to be of benefit (efficacy in a small population of patients) and has an acceptable safety profile (discovery and assessment of any adverse effects). Subsequently, safety and efficacy data are collected with informed consent, and a second application for approval is submitted.

5.2.4 | MFDS

Korea's EV industry and pharmacovigilance are still in the early stages of development. In 2018, the Korean MFDS issued guidelines on the quality, nonclinical, and clinical assessment of EV therapy products [65]. As the first document on EV regulation, the majority of the criteria outlined in the MISEV 2018 guidelines and the MFDS guidelines are notably similar. The MFDS guidelines provide detailed instructions on the characterization of starting materials and methods for the production, isolation, and characterization of EVs. These guidelines also cover stability testing, considerations for nonclinical studies, toxicological evaluation, and guidelines for clinical studies [66, 67].

6 | Conclusions

EV therapy is a rapidly advancing field that requires a nuanced regulatory framework because of the inherent complexity of EV products. Despite notable progress, no current EV-based therapies have received global regulatory approval. Key obstacles include ambiguity in defining EV compositions, a lack of consensus on review criteria across various jurisdictions, and the need for harmonized standards. A "one-size-fits-all" regulatory model is unlikely to be effective, and a case-by-case approach will be essential.

Regulatory challenges arise from the diverse nature of EVs and the limited understanding of their mechanisms of action. Critical factors such as biogenesis, isolation protocols, route of administration, dosing frequency and amounts, and therapeutic windows for EV administration must be better understood to ensure maximal efficacy without adverse effects. The variability of EV formulations, particularly injectables, further complicates the definition and control of active ingredients and impurities. Even for stem cell-derived EVs, the pharmacological mechanisms remain largely unclear, hindering the development of robust quality control frameworks.

The inconsistency of current regulatory strategies and the heterogeneity of EV production underscore the need for standardized production processes and harmonized quality control measures. Additionally, a coordinated global effort is necessary to establish industry-wide standards and potentially a centralized database of EV test reports. Regulatory authorities should also develop specialized guidelines focusing on cellular activity and drug mechanisms.

The translation of EV research into GMP-compliant therapies is essential for clinical applications. Key priorities include standardizing production methods, deciphering therapeutic mechanisms, resolving regulatory ambiguities, improving targeting, and mitigating immunogenicity through pharmacology and activity studies. Despite the challenges, interdisciplinary collaboration among stakeholders will be crucial for advancing clinical and industrial applications.

Effective quality control requires a deep understanding of the EV pharmacological mechanism of action and the material basis of their therapeutic function. Functional and efficacy assays must align with defined control objectives for QC approval. As the field evolves, advanced analytical tools are likely to play a key role in characterizing EV composition and purity, potentially leading to clearer regulatory oversight similar to that of biologics.

Author Contributions

Qiushi Li: conceptualization (lead), data curation (lead), formal analysis (lead), visualization (lead), writing – original draft (lead). **Yuxia Li:** conceptualization (equal), data curation (equal), formal analysis (equal), resources (equal). **Jianhua Sun:** data curation (supporting), formal analysis (supporting), resources (equal), supervision (equal). **Lan Hu:** data curation (equal), formal analysis (equal), investigation (equal), methodology (equal). **Xia Yun:** conceptualization (equal), investigation (equal), methodology (equal), resources (equal), software (equal). **Chen liuqing:** conceptualization (equal), methodology (equal), supervision (equal), validation (equal), visualization (equal). **Likun Gong:** conceptualization (equal), data curation (equal), formal analysis (equal), resources (equal), supervision (equal). **Shuxia Wu:** conceptualization (equal), funding acquisition (lead), project administration (equal), supervision (equal), visualization (equal), writing – review and editing (lead).

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The authors have nothing to report.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

Qiushi Li, Yuxia Li, and Jiaqing Shao are staff of AIE Bioscience (Guangdong) Co. Ltd., Torch Development Zone, Zhongshan, China. The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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