

# Principles and regulatory pathways of exosome-based products in wound healing: Insights from the Iranian Food and Drug Administration guideline

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## ARTICLE INFO

### Article type:

Review

### Article history:

Received: Dec 30, 2025

Accepted: May 31, 2026

### Keywords:

Biological products

Drug approval

Exosomes

Extracellular vesicles

Government regulation

Nanomedicine

Regenerative medicine

Wound Healing

## ABSTRACT

Wound healing is a critical clinical challenge, particularly for chronic and refractory wounds complicated by infection, necrosis, amputation, and death. Recently, cell derivatives, particularly extracellular vesicles (EVs) from mesenchymal stem cells, have emerged as promising cell-free therapeutic agents in regenerative medicine. The review aims to introduce small extracellular vesicles (sEVs) as a next-generation therapeutic strategy and to describe different EV types according to the Global Regulatory Framework (GRF). We also compare techniques for isolating, purifying, and characterizing sEVs and evaluate sEV-based therapies in tissue engineering, focusing on their scope and limitations. A narrative review of the literature was conducted, focusing on preclinical and clinical studies investigating EV-based therapies in wound healing, along with an investigation of the GRF, particularly the Iranian Food and Drug Administration (IFDA) guideline on EVs. The IFDA has recently established a framework for categorizing extracellular particles and highlighting the structural complexity and functional diversity of EVs. sEVs demonstrate significant therapeutic potential for wound repair by promoting angiogenesis, enhancing epithelialization, modulating inflammation, and accelerating tissue remodeling. To enhance the stability and therapeutic lifespan of sEVs, advanced delivery systems, including hydrogels, nanofibers, and biomatrices, are used to preserve structural integrity, physicochemical stability, biological functionality, and morphological characteristics, and to control their release at the injury site. EV-based therapies represent a promising and versatile approach in regenerative medicine for wound healing. Despite challenges in isolation, characterization, scalable manufacturing, and regulatory approval, sEV-based therapeutics offer a biocompatible, cost-effective, and versatile platform that bridges nanotechnology and regenerative medicine.

► Please cite this article as:

Nazari D, Heidari B, Akbari N, Soleymani Z. Principles and regulatory pathways of exosome-based therapies in wound healing: Insights from the Iranian Food and Drug Administration guidelines. Iran J Basic Med Sci 2026; 29: 1337-1354. doi: <https://dx.doi.org/10.22038/ijbms.2026.93213.20101>

## Introduction

The skin is the body's largest and most essential defensive barrier, protecting against external factors while regulating temperature and maintaining fluid balance, thereby supporting overall physiological stability. Despite its resilience, this primary barrier is frequently exposed to acute and chronic wounds. For decades, effective wound management, particularly for chronic and treatment-resistant ulcers, has remained a major challenge for specialists in regenerative medicine and reconstructive surgery (1).

Depending on wound type, severity, and patient-specific factors, wound management requires adherence to multistage therapeutic protocols, including debridement, infection control, maintenance of optimal moisture, and stimulation of tissue regeneration. The primary objective of these strategies is to accelerate tissue repair, alleviate pain, and prevent complications, thereby improving patient quality of life (2). However, conventional therapeutic strategies often fail to achieve complete tissue regeneration, particularly in chronic wounds such as diabetic ulcers and

pressure injuries, highlighting the need for more effective regenerative approaches.

Emerging technologies such as cell therapy have attracted significant attention in wound healing. However, their clinical translation remains limited due to several critical challenges (3). One of the major limitations of mesenchymal stem cell (MSC)-based therapies is their time-consuming, costly, and technically demanding preparation process, along with poor post-transplantation survival rates, where fewer than 1% of cells remain viable after two weeks (3). Furthermore, concerns regarding tumorigenicity, genetic instability, and uncontrolled differentiation continue to raise safety and ethical considerations, limiting widespread clinical application (4, 5). These limitations have prompted researchers to explore alternative regenerative strategies, particularly cell-free therapeutic approaches that use bioactive cellular derivatives rather than living cells (6). In this context, paracrine factors released by stem cells—including extracellular vesicles, growth factors, and other signaling molecules—have been recognized as key mediators of tissue

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repair and regeneration. Such approaches not only mitigate the risks associated with direct cell transplantation but also allow greater control over therapeutic composition and dosage.

Among these bioactive derivatives, exosomes (EXOs) are nanosized extracellular vesicles secreted by various cell types. These small extracellular vesicles (sEVs) carry a diverse cargo of proteins, lipids, mRNA, and microRNA that facilitate intercellular communication and modulate key biological processes involved in wound healing, including angiogenesis, inflammation regulation, and tissue remodeling (6). Their nanoscale size, natural biocompatibility, and ability to transfer bioactive molecules make them particularly attractive candidates for next-generation regenerative therapies. Compared with stem cell-based therapies, extracellular vesicle-based approaches offer several practical and biological advantages, including lower immunogenicity, improved safety profiles, easier storage, and greater potential for standardized manufacturing and scalable production (7-11). In addition, the defined molecular composition of sEVs enables more reliable quality control and reproducibility, which are essential for clinical translation and regulatory approval. Beyond their therapeutic applications, small nanosized extracellular vesicles have also attracted considerable attention as potential diagnostic biomarkers, since their molecular cargo reflects the physiological state of their parent cells. This cell-specific molecular signature enables early detection and monitoring of diseases such as cancer, inflammatory disorders, and cardiovascular conditions (7, 12-14).

Although numerous studies have explored the biological functions and therapeutic potential of sEVs in disease treatment and early diagnosis, there remains a lack of comprehensive reviews that simultaneously examine the clinical applications of EVs alongside relevant guidelines and regulatory frameworks, particularly in tissue engineering. In regenerative medicine, wound-healing products range from simple dressings to complex biological and medical products, including bandages, topical treatments, advanced wound-care devices, stem-cell products, and cell-free products such as EVs. Regulatory agencies establish stringent manufacturing standards to guarantee consistent quality and sterility. A clear articulation of the regulatory pathway underscores the critical importance of adhering to these guidelines, especially regarding the therapeutic applications of wound-healing interventions. Even post-approval, regulatory agencies continuously monitor product performance to detect any unforeseen safety issues or adverse events. In Iran, the Iranian Food and Drug Administration (IFDA) classifies these products based on their risk and intended use, which dictates the level of scrutiny and the specific evidence required for market authorization. The review aims to comprehensively detail and compare various types of extracellular vesicles, undertake a comparative examination of global regulatory frameworks, and present a perspective of the biological mechanisms and therapeutic applications of sEV-based therapies in wound healing. By integrating mechanistic insights, therapeutic evidence, and regulatory perspectives, this study seeks to provide a clearer translational roadmap for the development and clinical implementation of sEV-based therapies.

## Materials and Methods

### Study design

This manuscript presents a narrative review of sEV-based therapies in wound healing, with a specific focus on analyzing the regulatory pathways and global regulatory frameworks

established by the Iranian Food and Drug Organization. The review synthesizes existing knowledge on the therapeutic potential of sEVs for wound regeneration and examines the IFDA's framework for advanced biological products. The aim is to provide insights into the current landscape and regulatory considerations for sEV applications in this field, particularly in the Iranian context, while also comparing with international regulatory standards.

### Literature search strategy

A comprehensive literature search was performed to identify relevant scientific publications and regulatory documents. The primary databases searched included PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search strategy combined keywords related to sEVs, wound healing, and regenerative medicine with terms pertaining to regulatory affairs and specific authorities. Keywords such as "extracellular vesicles", "wound healing", "skin regeneration", "therapeutic applications", "manufacturing", "characterization", "Iranian Food and Drug Organization", "IFDA guideline", "biologics regulation", "Food and Drug Administration", and "European Medicines Agency" were utilized. Boolean operators (AND, OR) and wildcard characters were employed to optimize search sensitivity and specificity. The search was conducted up to 2026. Furthermore, reference lists of key review articles and relevant publications were manually screened to identify any additional pertinent studies that the initial database search might have missed. The IFDA guideline on extracellular vesicles was accessed directly from the official IFDA website.

### Inclusion and exclusion criteria

Studies were included if they were peer-reviewed articles, original research papers, or relevant review articles that discussed extracellular vesicle applications in wound healing or tissue regeneration, and/or addressed regulatory aspects of such therapies. Publications were considered eligible if they provided information on the biological mechanisms, therapeutic efficacy, manufacturing processes, characterization methods, or regulatory requirements for EV-based products. Both *in vitro* and *in vivo* studies, as well as preclinical and early clinical investigations, were considered. Regulatory documents and global guidelines from IFDA, US FDA, EMA, and ICH were also included. Articles were excluded if they were not published in English or Persian, were conference abstracts with insufficient content, or focused solely on other regenerative therapies without a significant vesicle component. Non-peer-reviewed literature and opinion pieces were also excluded.

### Study selection and data synthesis

The literature search results were initially screened by title and abstract to identify potentially relevant articles. Full texts of the selected articles were then retrieved and reviewed independently to confirm eligibility based on the predefined inclusion and exclusion criteria. Discrepancies in study selection were resolved through discussion among the authors to reach a consensus. The data extracted from the included studies encompassed information on exosome sources; isolation and purification methods (e.g., ultracentrifugation, density gradient centrifugation, tangential flow filtration); characterization techniques (e.g., NTA, TEM, Western blot); therapeutic outcomes in wound-healing models; and details on regulatory guidelines and requirements. Synthesis of the information involved a thematic approach, categorizing findings into sections covering the biological basis, therapeutic applications, manufacturing and characterization challenges, and the

regulatory landscape, with a dedicated focus on the IFDA guideline and its comparison with international standards.

### Regulatory document analysis

The IFDA guideline on extracellular vesicles was systematically reviewed to identify key recommendations regarding the development, manufacturing, and quality control of sEV-based therapeutics. This analysis focused on aspects such as product definition, recommended analytical methods for characterization (e.g., particle size, concentration, purity, potency), manufacturing process controls, and safety assessment. Comparative analysis was performed by cross-referencing the IFDA requirements with those outlined in relevant guidelines from the Federal Food, Drug, and Cosmetic (FD&C), European Medicines Agency (EMA), Ministry of Health, Labour and Welfare (MHLW) and Pharmaceuticals and Medical Devices Agency (PMDA) of Japan, The Ministry of Food and Drug Safety (MFDS) of the South Korea, Taiwan Food and Drug Administration (TFDA). This comparative approach aimed to identify areas of alignment, divergence, and potential challenges in navigating international regulatory expectations for sEV-based products.

### Extracellular vesicles (EVs)

Among cell-derived vesicles, extracellular vesicles are valuable nanostructures with substantial therapeutic potential. The Iranian Food and Drug Administration, in its latest guideline numbered GUIDPNABIO012 (August 26, 2025), has introduced specialized classifications and definitions for cell-secreted vesicles. According to this guideline, extracellular vesicles are defined as biological nanoparticles enclosed by a lipid bilayer and lacking independent replicative capacity. This definition is largely consistent with internationally accepted descriptions of EVs. For instance, the International Society for Extracellular Vesicles (ISEV) defines EVs as lipid bilayer-enclosed particles released by cells that cannot replicate and carry bioactive molecules involved in intercellular communication. Similarly, regulatory authorities such as the US FDA and the European Medicines Agency consider EV-based therapeutics within the broader category of biological medicinal products or advanced therapy medicinal products (ATMPs), depending on their origin and manufacturing processes. Although these international frameworks do not provide a detailed subclassification of EVs comparable to the IFDA system, they emphasize strict requirements for identity, purity, potency, safety, and manufacturing consistency, which are also reflected in the IFDA guideline.

From a molecular perspective, EVs contain five distinct protein groups. The first and second groups serve as universal markers for identifying and evaluating the overall structural and functional characteristics of EVs. These include membrane-associated or glycosylphosphatidylinositol (GPI)-anchored proteins linked to the plasma membrane or endosomes. Within these groups, both multi-pass transmembrane proteins and single-pass transmembrane proteins are detected. Tetraspanins (CD9, CD63, CD81, and CD82) and other transmembrane proteins such as CD47 and TSAP6 are classified as multi-pass transmembrane proteins. Single-pass transmembrane proteins include MHC-I and MHC-II molecules, ITGA/ITGB, TFR2, LAMP1/2, as well as heparan sulfate proteoglycans (syndecans, EMMPRIN, ADAM10) and GPI- or lipid-anchored proteins (e.g., glypicans, CD73 nucleotidase, and complement-binding protein CD59). Both single- and multi-pass transmembrane proteins are expressed across EV populations and include

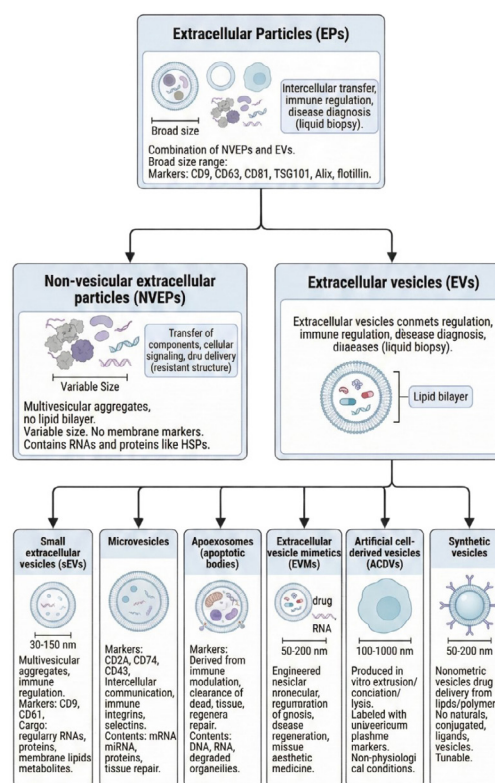
lipid- or membrane-binding proteins such as CHMP (ESCRT-I/II/III components, e.g., TSG101), accessory proteins (e.g., syntenin/SDCBP, caveolins/CAV, flotillins/FLOT1/2, ARDC1, VPS4A/B, ALIX/PDCD6IP), heat shock proteins (HSC70, HSPA8, HSP90AB1/HSP84), and cytoskeletal proteins (actin/ACT, tubulin/TUB, GAPDH). Proteins in the third group are used to assess EV purity relative to common contaminants. The fourth group comprises membrane-associated, lipid-bound, and soluble proteins linked to intracellular compartments other than endosomes or the plasma membrane. Together with the fifth group, these provide additional insights into the probable intracellular origin of EVs or co-isolated components. Finally, the fifth group includes secreted proteins recovered alongside EVs during isolation (15).

Importantly, similar molecular markers have also been recommended in international EV characterization guidelines, such as Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV 2018), which emphasize the use of tetraspanins (CD9, CD63, CD81), ESCRT-associated proteins (TSG101, ALIX), and cytoskeletal proteins as key indicators for EV identification. The convergence of these markers across IFDA and international recommendations reflects a growing consensus regarding the minimal criteria required for EV characterization and quality control.

There are two specialized systems for classifying EVs. In one framework, EVs are categorized into several vesicle types (Figure 1), a classification approach that is consistent with the principles outlined in the IFDA guideline.

### Extracellular particles (EPs)

A broader category encompassing both NVEPs and EVs. These extracellular entities are secreted under physiological conditions by various cell types (e.g., neurons, immune cells, cancer cells), as well as in pathological states (e.g.,



**Figure 1.** Classification of extracellular particles according to Iranian Food and Drug Administration (IFDA, GUI-DPNA-BIO-012, 2025)

inflammation, cancer, neurological disorders) (16).  
*Non-vesicular extracellular particles (NVEPs)*

Multivesicular aggregates released from cells that lack a surrounding lipid bilayer (Table 1) (16).

**Table 1.** Morphological characteristics, biochemical properties, and biogenesis of extracellular vesicles according to the IFDA guideline (GUI-DPNA-BIO-012) (21)

| Type                                          | Source                                                                                                                                                                                              | Size                                               | Function                                                                                                                                                                                                                                                                               | Markers                                                                                                                                                                                           | Contents                                                                                                                                                        |
|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Non-vesicular extracellular particles (NVEPs) | Extracellular particles secreted from cells without the formation of classical vesicles and lacking a lipid bilayer                                                                                 | Variable; no defined vesicular structure           | Transfer of biological components such as proteins and RNAs; cellular signaling; drug delivery due to resistant structure and minimal immune activation                                                                                                                                | Lacking classical membrane markers                                                                                                                                                                | Multivesicular aggregates without a phospholipid bilayer, containing RNAs, dispersed cellular components, and specific proteins such as HSPs or non-coding RNAs |
| Extracellular particles (EPs)                 | Combination of NVEPs and EVs. Under physiological conditions by various body cells; under pathological conditions following inflammation, cancer, neurological diseases, etc.                       | Broad range, depending on constituent components   | Intercellular transfer of biological information, intercellular interactions, immune response regulation, disease diagnosis via liquid biopsy, loading of drugs, RNAs, proteins, or therapeutic RNAs; role in progression of certain diseases (e.g., cancer, Alzheimer's, Parkinson's) | Dependent on constituent particle type (EVs: CD9, CD63, CD81, TSG101, Alix, flotillin) (NVEPs: lacking classical membrane markers; may contain specific proteins such as HSPs or non-coding RNAs) | Combination of NVEPs and EVs, including RNA, protein, lipid, and specific proteins such as HSPs or non-coding RNAs                                              |
| Extracellular Vesicle Mimetics                | Via direct cellular manipulation using bioengineering or physical methods such as mechanical extrusion, sonication, lipid nanoparticle engineering, polymer integration, or biomolecule conjugation | Similar to sEVs (30–150 nm)                        | Simulation of EV function in therapy and drug delivery                                                                                                                                                                                                                                 | Dependent on production method, may resemble EVs                                                                                                                                                  | Customizable; includes drugs, RNA, targeted proteins                                                                                                            |
| Artificial cell-derived vesicles (ACDVs)      | <i>In vitro</i> from cells under non-physiological conditions via extrusion, sonication, or cell lysis                                                                                              | 100–1000 nm                                        | Transfer of cellular components under non-physiological conditions                                                                                                                                                                                                                     | May include plasma membrane markers, cytoplasmic components, proteins, lipids, and RNA                                                                                                            | —                                                                                                                                                               |
| Synthetic vesicles                            | Synthetic vesicles constructed de novo or as hybrids with EVs. Via sonication, extrusion, or coacervation                                                                                           | Tunable; typically, nanometric (usually 50–200 nm) | Targeted drug delivery, vaccine development, and simulation of EV function                                                                                                                                                                                                             | No natural surface markers, but can be purposefully conjugated with molecules such as ligands, antibodies, or cell-penetrating peptides to enhance function and targeting                         | Drugs, RNA, specific proteins, and synthetic lipids                                                                                                             |
| Small operational EVs                         | Derived from cells; subset of EVs                                                                                                                                                                   | <150 nm                                            | Intercellular transfer of biological information, regulation of cellular activities, targeted therapies, disease diagnosis, and biomedical research                                                                                                                                    | CD9, CD63, CD81, TSG101, Alix                                                                                                                                                                     | Regulatory RNAs such as miRNA and siRNA, signaling proteins, membrane lipids, and metabolites vary by source cell and physiological condition                   |
| ApoExos (apoptotic bodies)                    | Vesicles derived from programmed cell death (apoptosis)                                                                                                                                             | 500–1000 nm diameter                               | Immune response modulation, clearance of dead cells, occasional stimulation or suppression of inflammation, tissue regeneration                                                                                                                                                        | CD9, CD63, CD81, and apoptosis-related markers such as Annexin V and phosphatidylserine                                                                                                           | Proteins, DNA, RNA, degraded organelles, lipids, miRNA, cell death-associated proteins, cellular antigens, and inflammatory molecules                           |
| Microvesicles                                 | Detached from the cell surface in response to physiological or pathological stimuli (e.g., cellular stress, inflammation, activation, or apoptosis) via membrane blebbing                           | 100–1000 nm                                        | Transfer of biological molecules such as RNA, proteins, lipids, and cytokines to target cells; regulation of inflammatory responses                                                                                                                                                    | CD29, CD44, CD73, CD40, phosphatidylserine, integrins, selectins                                                                                                                                  | mRNA, miRNA, signaling proteins, lipids, cytokines                                                                                                              |

### Extracellular vesicles

EVs are defined as small lipid bilayer-enclosed biological particles naturally secreted by cells that cannot replicate but serve as dynamic carriers of proteins, lipids, and nucleic acids, enabling communication between cells. By mediating intercellular communication, EVs play vital roles in regulating immune responses, tissue repair, and disease progression, while also offering significant potential as biomarkers and therapeutic tools for diagnostics and targeted drug delivery (16).

### Extracellular vesicle mimetics (EVMs)

Vesicle-like particles generated through direct artificial manipulation, also referred to as extracellular vesicle mimetics. These engineered nanostructures replicate the biological properties, function, and morphology of natural EVs. Typically ranging from 50 to 200 nm, they can encapsulate RNA, therapeutic proteins, or targeted drugs, depending on the fabrication method. EVMs may be derived from live cells (e.g., stem or immune cells) or synthetic constructs (e.g., liposomes). They facilitate precise molecular delivery, tissue regeneration, and immune modulation. Surface markers (e.g., CD9, CD63, and CD81) are tunable based on origin and targeting, enabling specific cellular interactions. Owing to these features, EVMs hold significant promise in advanced therapeutics, targeted drug delivery, and aesthetic medicine (Table 1) (16).

### Artificial cell-derived vesicles (ACDVs)

Mimetic vesicles produced *in vitro* from various cell types using physical or chemical methods such as extrusion, sonication, or cell lysis. Because they are generated under nonphysiological conditions, regulatory agencies—including the US FDA and the EMA—typically evaluate them alongside biologically derived nanomedicine or engineered-vesicle products, requiring detailed characterization of production methods and safety profiles.

### Synthetic vesicles (SVs)

Nanometric spherical structures synthesized *de novo* from molecular components or as hybrids (e.g., liposome-EV fusion). Composed of lipid bilayers or polymeric membranes, SVs are fabricated via sonication, extrusion, or coacervation. Their primary role is precise delivery of biologics (e.g., drugs, RNA, proteins, antigens). With tunable size, membrane composition, and cargo, SVs find applications in targeted drug delivery, vaccine development, cancer therapy, and biotechnology, including artificial biology for simulating cellular components (Table 1) (16).

### Small extracellular vesicles

Operationally defined as nanoparticles <150 nm (typically 30–150 nm) secreted by cells. sEVs are essential mediators of intercellular communication and information transfer. They carry characteristic markers such as CD9, CD63, CD81, TSG101, and Alix, and their cargo includes regulatory RNAs (miRNA, siRNA), signaling proteins, membrane lipids, and metabolites. Owing to their small size and bioactive content, sEVs are widely explored in targeted therapy, diagnostics, and biomedical research (Table 1) (16).

### Apoexosomes (apoptotic bodies or ApoExos)

Vesicles derived from the late stages of programmed cell death (apoptosis). They are marked by Annexin V and

phosphatidylserine and encapsulate proteins, lipids, DNA, RNAs, chromatin, and degraded organelles. Apoexosomes mediate intercellular communication, enhance phagocytosis, regulate immunity, eliminate viruses, protect vasculature, promote regeneration, and aid diagnostics. As drug carriers, they improve stability and efficacy. Reports indicate promising potential in the diagnosis, prognosis, and treatment of cancers, inflammatory diseases, cardiovascular disorders, and tissue repair (17).

### Microvesicles (MVs)

MVs derived from the plasma membrane, typically ranging from 100 to 1000 nm in diameter. While their formation mechanisms resemble those of sEVs, the primary distinction lies in membrane origin. Unlike endosome-derived sEVs, MVs bud directly from the plasma membrane. They express markers including CD29, CD44, CD73, CD40, integrins, and selectins. MVs production is elevated in pathological conditions, including acute coronary syndrome, hypertension, inflammatory/autoimmune diseases, infections, and cancer. Upon release, they carry cytoplasmic components, including cytokines, signaling proteins, miRNAs, and mRNAs, whose composition depends on the cell source and environment. MVs may remain near the site of release or circulate in biofluids, transferring signals to distant cells. Functionally, they contribute to intercellular communication, immune regulation, inflammation, and tissue repair. Microvesicles are gaining recognition as biomarkers and targeted delivery vehicles in cancer, cardiovascular, and neurological research (Table 1).

Another specialized system classifies extracellular vesicles into three main categories: exosomes, exomers, and ectosomes (18). Ectosomes refer to vesicles of plasma membrane origin (not endosomal) that are released directly from the cell surface rather than from intracellular membrane-bound structures or organelles. They represent a broad population of extracellular vesicles ranging in size from 50 nm to 1  $\mu$ m. Exomers are non-membrane-bound nanovesicles, approximately 50 nm in diameter, with a distinct structure compared to other EV categories. These nanovesicles are primarily studied in basic research on molecular transfer and biological nanoparticles, and have also recently found diverse applications in nanotechnology, particularly in the design and development of nanocarriers for drugs or biosensors. The most clinically relevant group in this classification is exosomes. EXOs are nano-sized extracellular vesicles of endosomal origin with an intraluminal diameter of 40–160 nm and a density of 1.13–1.19 g/mL. They arise from multivesicular bodies, discrete intracellular membrane-bound structures, and play critical roles in intercellular communication, diagnostics, and therapeutic applications (18).

Overall, in accordance with the ISEV guidelines, the first classification scheme is recommended. This guideline recommends using the general term “EVs” instead of exosomes or ectosomes, and defining their subtypes more specifically by adding appropriate prefixes to “EVs”. These prefixes can be selected based on size, composition, molecular composition, or cell origin. For example, exosomes are referred to as sEVs, while ectosomes are referred to as medium-sized extracellular vesicles (mEVs).

### Exosomes or sEVs

The term **exosome** was first introduced in 1987 to

describe small membrane vesicles released from cells during reticulocyte maturation (19). Exosomes are nanosized, cupshaped sEVs surrounded by a lipid bilayer membrane, typically ranging from 30 to 150 nm in diameter. These vesicles are generated via the endosomal pathway and are released upon fusion of multivesicular bodies with the plasma membrane (20). They are characterized by a range of protein and lipid markers involved in their biogenesis and trafficking. Common protein markers in EXOs identification include membranefusion-related proteins such as GTPases, annexins, and flotillins, as well as Rab family proteins and tetraspanins, including CD9, CD63, CD81, and CD82 (25, 26). Additional surface molecules, such as CD11b and CD54, have also been reported on exosomal membranes. The lipid composition of EXOs includes cholesterol, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, and hexosylceramides, which contribute to membrane stability and vesicle formation (21). Exosomal membranes are typically enriched in saturated fatty acids and cholesterol, features that distinguish them from other cellular membranes.

Recent studies have identified additional markers associated with the endosomal origin of EXOs, including lysosomeassociated membrane proteins (LAMP1, LAMP2, and LAMP3) and proteins such as CD107a, CD107b, CD208, and TSG101, which facilitate differentiation of

exosomes from other extracellular vesicle subtypes (22). The presence of these molecules supports the endosomal biogenesis of exosomes and highlights their essential role in intercellular communication. In particular, sEVs released by antigenpresenting cells may contain major histocompatibility complex class II (MHCII) molecules, thereby modulating immune responses and antigen presentation pathways (23). A defining feature of stem cellderived exosomes is their complex molecular cargo, which includes a diverse range of proteins, lipids, nucleic acids, amino acids, metabolites, and other bioactive macromolecules. These vesicles function as biological carriers that transfer regulatory molecules between cells. To date, more than 850 gene products and over 150 microRNAs have been identified within exosomal cargo, reflecting their extensive functional diversity (24).

Recent analyses have also highlighted the heterogeneity of EXOs in size, molecular content, cellular origin, and biological function (Table 2). Based on size distribution, EXOs have been categorized into three subgroups: ExoA (40–75 nm), ExoB (75–100 nm), and ExoC (100–160 nm). Classification based on molecular content has also been proposed, with Exo1, Exo2, and Exo3 differing in their surface markers and cargo composition. For example, Exo1 vesicles typically contain CD63 together with nucleic acids and specific protein cargo, whereas Exo2 and Exo3 are characterized by CD9 and CD81, respectively, along with

**Table 2.** Classifications of exosomes based on size, content, origin, and function

| Classification | Subgroup     | Size (nm) | Biomarkers                                    | Molecular contents                                                                                                                                 | Biological function                                                                               | Cellular origin                                                                              |
|----------------|--------------|-----------|-----------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| Size           | ExoA         | 40–75     | CD63, CD81                                    | Typically enriched in regulatory miRNAs, heat shock proteins such as HSP70, and tetraspanins                                                       | High tissue penetration; rapid information transfer due to small size                             | Diverse (condition-dependent)                                                                |
|                | ExoB         | 75–100    | CD9, CD81                                     | Balanced combination of miRNAs and messenger mRNAs, membrane proteins, possibly containing HSP70 and TSG101                                        | Cargo-carrying capacity; regulation of growth and cellular communication                          | Diverse                                                                                      |
|                | ExoC         | 100–160   | CD9, Flotillin-1                              | Likely containing larger proteins, DNA, lipids, and biogenesis pathway markers such as Alix                                                        | Higher capacity for transporting large molecules                                                  | Diverse                                                                                      |
| Content        | Exo1         | —         | CD63, Protein X                               | Membrane proteins, miRNA, metabolic enzymes                                                                                                        | Regulation of cell survival and intercellular communication                                       | Immune and epithelial cells                                                                  |
|                | Exo2         | —         | CD9, Protein Y                                | Messenger RNAs, signaling proteins                                                                                                                 | Regulation of growth and cellular differentiation                                                 | Stem cells, cancer cells                                                                     |
|                | Exo3         | —         | CD81, Protein Z                               | Adhesion proteins, miRNA, lipids                                                                                                                   | Regulation of immune response and cell migration                                                  | Hepatic and brain cells                                                                      |
| Origin         | ExoI         | —         | CD63, HSP70, TSG101, Annexin A2               | Contains anti-apoptotic proteins such as Bcl-2, heat shock proteins HSP70 and HSP90, and survival-regulating miRNAs                                | Induction of cell survival and stress resistance                                                  | Brain origin                                                                                 |
|                | ExoII        | —         | CD9, Alix, apoptotic proteins such as Bax     | Apoptotic proteins such as Bax and Caspase-3, growth-suppressing miRNAs, and inflammatory factors such as TNF- $\alpha$                            | Induction of cell death and growth regulation                                                     | Cancer origin                                                                                |
|                | ExoIII       | —         | CD81, MHC class I and II, Flotillin-1, ICAM-1 | Enriched in MHC class I and II antigens, cytokines such as IL-6 and IL-10, immune response-regulating miRNAs, and adhesion proteins such as ICAM-1 | Regulation of immune response and inflammation                                                    | Hepatic origin                                                                               |
|                | Exo $\alpha$ | —         | CD9, CD63, CD81, TSG101, Annexin A1           | Anti-apoptotic miRNAs, growth factors, anti-inflammatory proteins, and regulatory lipids                                                           | Enhancement of cell survival and viability; support for cellular persistence and stress reduction | Mesenchymal stem cells, epithelial cells, and repairing neural cells                         |
| Function       | Exo $\beta$  | —         | FasL, TRAIL, TNF, CD63                        | Cell death-inducing proteins, caspases, and death-regulating RNAs                                                                                  | Induction of apoptosis and elimination of damaged or cancerous cells                              | Activated immune cells, stressed cancer cells                                                |
|                | Exo $\gamma$ | —         | CD81, CD86, MHC class I and II                | Cytokines, immune-regulating RNAs, cellular antigens, immunomodulatory proteins                                                                    | Immune system modulation and regulation of immune responses                                       | Dendritic cells, macrophages, Treg cells, and epithelial cells under inflammatory conditions |

distinct molecular contents.

Exosomal heterogeneity may also arise from differences in tissue origin, leading to categories such as ExoI, ExoII, and ExoIII, which originate from brain, pancreatic, and hepatic cells, respectively. In addition, functional classifications have been proposed based on biological activity: Exo $\alpha$ , which promotes cell survival and viability; Exo $\beta$ , which can induce apoptosis; and Exo $\gamma$ , which modulates immune responses across multiple cell types. These variations in composition and surface receptor expression may significantly influence the interaction of exosomes with recipient cells. Functionally, the macromolecular components of EXOs play critical roles in regulating diverse biological processes. Exosomes contribute to immune modulation, inflammation management, angiogenesis promotion, and apoptosis regulation, and they are increasingly being investigated as therapeutic tools in regenerative medicine. Recent studies have also highlighted their potential applications in neurological disorders, cancer therapy, and tissue repair, including wound healing (Figure 2) (25).

#### Isolation and purification of exosomes: Principles and techniques

Exosome isolation remains technically challenging because of their small size (30–150 nm), low density (1.13–1.19 g/ml), and molecular heterogeneity, as well as the presence of contaminants such as proteins, lipoproteins, and cellular debris. Current isolation techniques often struggle to fully separate EXOs from lipoproteins with similar physicochemical properties, or from extracellular vesicles originating from non-endosomal pathways, thereby compromising sample purity. Several techniques have been developed for the enrichment and purification of extracellular vesicles (26–30). The selection of an appropriate isolation method depends on multiple factors, including sample volume, downstream applications, required purity, and scalability. For small-volume samples, ultracentrifugation in sterile tubes remains a commonly used method for separating vesicles by density and size. However, scalability remains a major limitation for many conventional isolation techniques. Alternative methods, such as tangential flow filtration (TFF), enable scalable

and nonspecific concentration and purification of extracellular vesicles. When combined with diafiltration, vesicle preparations can be efficiently washed through buffer exchange. Diafiltration also facilitates formulation development by enabling the incorporation of excipients or final-formulation buffers, such as cryoprotectants, that improve vesicle stability during storage (31, 33).

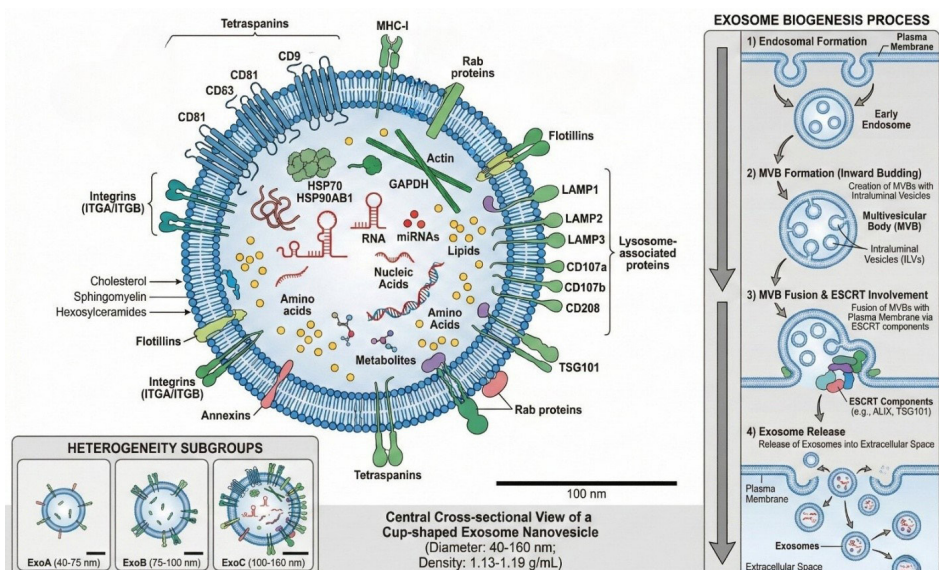
To ensure quality and reproducibility during extracellular vesicle purification, it is generally recommended to assess particle:protein ratios (particles/ $\mu$ g protein) and particle-size distribution before and after purification. Figure 3 summarizes commonly used exosome isolation methods and compares their respective advantages and limitations. Because different methods yield them with varying purity and recovery rates, careful selection of isolation techniques is essential for downstream experimental and therapeutic applications.

#### Ultracentrifugation

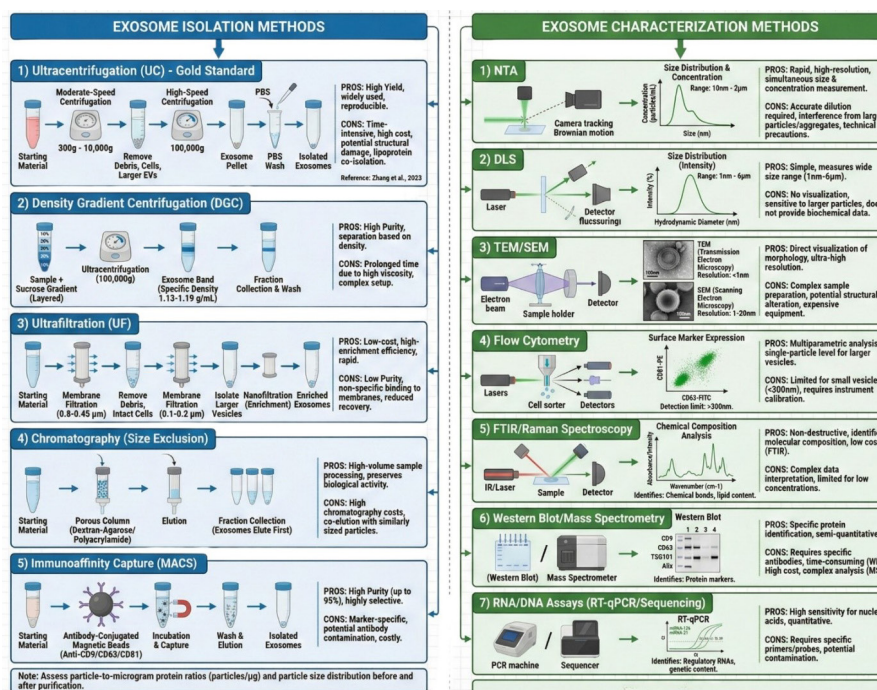
Ultracentrifugation is one of the most widely used techniques for exosome isolation and is often regarded as the gold standard for extracellular vesicle separation. This method separates dissolved particles according to differences in size and density. Théry et al. first applied ultracentrifugation to isolate exosomes from reticulocyte culture medium. The process typically involves two stages. Initially, moderate-speed centrifugation steps remove dead cells, cellular debris, and larger extracellular vesicles. Subsequently, EXOs are pelleted at high centrifugal forces ( $\sim 100,000 \times g$ ), followed by washing with phosphate-buffered saline (PBS) to remove contaminating proteins. Several parameters—including centrifugation time, applied force, rotor type, and environmental conditions—significantly influence the yield and purity of exosomes. Despite its widespread use, ultracentrifugation has several limitations, including high equipment costs, potential structural damage to vesicles, long processing times, and frequent coisolation of lipoproteins (32).

#### Density gradient centrifugation (DGC)

Density gradient centrifugation is frequently combined with ultracentrifugation to improve the purity of exosomes. This technique separates vesicles based on their buoyant



**Figure 2.** Composition and biogenesis of exosomes



**Figure 3.** Exosome isolation and characterization techniques

density within a density gradient medium, commonly sucrose or iodixanol. Although DGC significantly enhances EXO purity, the high viscosity of sucrose solutions slows vesicle sedimentation, thereby increasing processing time and making the procedure more laborintensive (33).

#### Ultrafiltration (UF)

Ultrafiltration employs membranes with defined pore sizes to achieve selective separation of cellular derivatives, including exosomes. Larger pores (0.8–0.45 µm) are used to remove intact cells and debris, whereas intermediate pores (0.1–0.2 µm) allow separation of larger vesicles. A final nanofiltration step enriches nanoscale vesicles such as EXOs. Ultrafiltration accounts for approximately 20% of EV isolation applications and is often used as a complementary technique. This method is relatively inexpensive and can achieve high enrichment efficiency without significantly affecting vesicle activity. However, challenges include membrane clogging, nonspecific binding of exosomes to filtration membranes, and reduced recovery rates (34).

#### Chromatography

Chromatographic techniques separate exosomes based on size or molecular interactions within porous matrices. In size exclusion chromatography (SEC), larger macromolecules are excluded from gel pores and elute earlier, whereas smaller particles enter the pores and elute later. This method preserves the biological activity of EXOs and enables relatively gentle isolation. Common chromatographic matrices include dextranagarose, polyacrylamide, and allyl dextran. While chromatography allows the processing of relatively large sample volumes, coelution of similarly sized particles may reduce purity, and the technique can be relatively expensive (35).

#### Immunoaffinity capture

Immunoaffinity capture isolates exosomes by exploiting antibody–antigen interactions between exosomal surface

markers and specific antibodies immobilized on beads or chromatographic supports. Antibodies targeting tetraspanins such as CD9, CD63, and CD81 are commonly used. This technique enables highly specific isolation of exosome subpopulations and is particularly useful for diagnostic or biomarker studies. However, its limitations include high cost, limited scalability, and the potential loss of vesicle subtypes that lack the targeted markers (36).

#### Characterization of exosomes

Characterization of exosomes requires evaluation of their structural, morphological, chemical, and biological properties. Each analytical method provides distinct advantages for assessing specific aspects of vesicle composition and function. However, individual techniques also have inherent limitations that must be considered to ensure accurate interpretation of results. International guidelines, such as MISEV, recommend combining multiple complementary methods—including size analysis, morphological imaging, and protein marker detection—to achieve reliable extracellular vesicle characterization (37). Table 3 summarizes key analytical techniques used for EXOs characterization along with their advantages and limitations.

#### Fourier transform infrared spectroscopy (FTIR)

FTIR is a widely used spectroscopic technique for identifying chemical bonds and functional groups in organic and inorganic materials. The method analyzes infrared radiation absorbed by the sample to produce a spectrum representing molecular vibrations. FTIR is particularly useful for studying surface chemistry and molecular composition of nanoparticles, including extracellular vesicles. Its advantages include low cost, rapid analysis, and minimal sample preparation, making it applicable across multiple biomedical and industrial fields (38, 39).

#### Nanoparticle tracking analysis (NTA)

NTA is commonly used to determine particle size

**Table 3.** Technical considerations and IFDA guideline for exosome characterization methods

| Analysis                                          | Methods                                                                                                                                                                                                                | Objective                                                                                                                                                                                                                                                                                                                              | Applications                                                                                                                                                                                                                                                        | Limitations / Key notes                                                                                                                                                                                                                                                                                                                            |
|---------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Particle quantification and hydrodynamic diameter | NTA, DLS, TRPS                                                                                                                                                                                                         | Determine sample parameters (e.g., Concentration, Media) and method-specific settings/data processing (e.g., Laser wavelength, Concentration range, Camera level, Threshold value, Temperature, Video duration)                                                                                                                        | Particle quantification in product development, in-process control, pharmaceutical analysis, stability studies, and final product analysis                                                                                                                          | NTA is less susceptible to interference from aggregates or larger particles compared to DLS. Validated for nanoparticle sizing per quality criteria if particles >50 nm                                                                                                                                                                            |
| Physical structure detection (exosome size)       | TEM                                                                                                                                                                                                                    | Examine the precise structure and actual nanoparticle diameter                                                                                                                                                                                                                                                                         | Differentiate EVs from non-vesicular particles                                                                                                                                                                                                                      | TEM is currently the most reliable imaging method for assessing the physical structure of nanoparticles and distinguishing EVs from non-vesicular particles. Complements NTA                                                                                                                                                                       |
| Surface Charge                                    | Zeta Potential                                                                                                                                                                                                         | Evaluate the surface electrical charge of EV molecules, dispersion, and particle suspension stability, aiding in understanding EV aggregation                                                                                                                                                                                          | Evaluate EV suspension stability                                                                                                                                                                                                                                    | Monitoring zeta potential is critical for assessing EV stability during product development. EV zeta potential is typically slightly negative                                                                                                                                                                                                      |
| Total protein quantification and purity           | Lowry, Biuret, BCA, Bradford, Fluorimetric-based assays                                                                                                                                                                | Determine the quantity and purity of proteins                                                                                                                                                                                                                                                                                          | Composition analysis, quality control                                                                                                                                                                                                                               | Combine Size Exclusion Chromatography (SEC) with UV detection at 280 nm for separation without chemical interactions. Quantify total protein via colorimetric assays; assess purity indirectly via particle-to-protein ratio (particles/ $\mu$ g)                                                                                                  |
| Total lipid quantification                        | Attenuated total reflection Fourier-transform infrared spectroscopy, Sulfo-phospho-vanillin assay (SPVA)                                                                                                               | Determine total lipid content. Less critical than protein quantification for exosomal content                                                                                                                                                                                                                                          | Optional in the product development phase                                                                                                                                                                                                                           | Optional in product development due to high exosome volume requirements and low lipid detection sensitivity                                                                                                                                                                                                                                        |
| Immunochemical characterization                   | Western blot, High-performance tandem Mass spectrometry, small particle flow cytometer, Exoview, MACSPlex, Nanoflow cytometry                                                                                          | Identify surface and internal exosomal molecular markers (key surface markers: tetraspanins like CD9, CD63, CD81; internal: TSG101, Alix, HSP70; supplementary: Annexin A2, Flotillin-1). Traceable via specific antibodies in immunocytochemistry; widely used in diagnostic/research studies on exosome function and cellular origin | Quality assessment, origin, purity of exosomes; drug substance testing, stability studies, final exosome product analysis                                                                                                                                           | Western blotting is an older immunochemical method that requires large EV quantities and extensive processing. High-performance tandem MS can replace it, identifying a protein panel (700–2000 proteins). Per MISEV guideline, verify expression of positive protein markers (at least categories 1 and 2) in EV-based products (others optional) |
| DNA content assay                                 | Capillary electrophoresis, Spectrophotometer, Nanodrop                                                                                                                                                                 | Identify the genetic information transferred to the target cells                                                                                                                                                                                                                                                                       | Non-invasive disease detection (e.g., cancers via liquid biopsy), mutation monitoring, tumor heterogeneity assessment, horizontal gene transfer mechanisms, treatment response prediction, disease recurrence monitoring, and new therapeutic target identification | DNA in exosomal content may be considered an impurity. Analyze DNA with/without DNase treatment for accurate detection                                                                                                                                                                                                                             |
| RNA content assay                                 | Nuclear RNAs (snRNA, snoRNA, scaRNA) via RT-qPCR, RNA-seq, Northern blot; Cytoplasmic RNAs (tRNA, miRNA, Y-RNA, mRNA, SRP-RNA, rRNA, lncRNA, piRNA) via RNA sequencing, specific microarrays, Bioanalyzer, TapeStation | Identify RNA types and functions to better understand intercellular communication, disease detection, and targeted therapy design                                                                                                                                                                                                      | Biomarker identification for cancers, neurological/inflammatory diseases; RNA-based drug development and novel therapy efficacy evaluation                                                                                                                          | RNA in exosomal content may be considered an impurity. Use RNase treatment for precise detection                                                                                                                                                                                                                                                   |

distribution and concentration of exosomes in the range of 10 nm to 2  $\mu$ m. The technique tracks the Brownian motion of individual particles suspended in liquid, enabling particle-by-particle analysis. NTA provides information on particle size, size distribution, concentration, and phenotype. It offers several advantages, including rapid measurement, simple sample preparation, and the ability to detect fluorescently labeled exosomal markers. However, accurate measurements require appropriate dilution and careful calibration. Additionally, fluorescent signal detection may be limited if emitted signals are weak or unstable (40).

#### Dynamic light scattering (DLS)

DLS serves as an alternative for exosome size detection, measuring particles ranging from a few nanometers to micrometers. It provides the diameter range of analyzed vesicles but offers no biochemical data or information on cellular origin. The method is relatively simple, but it does not visualize particles (40). Its advantage lies in its ability to measure particles from 1 nm to 6  $\mu$ m. However, even small amounts of larger vesicles in suspension can complicate the detection of smaller particles. Currently, the Horiba 100SZ device (Japan) is used for determining nanoparticle size and diameter in cellular derivatives via DLS. This instrument identifies particles from 0.3 nm to 10,000 nm with  $\pm 0.2\%$  accuracy, provides volume percentage (% Volume) and number percentage (% Number) graphs (41).

#### Transmission electron microscopy (TEM)

TEM is a powerful technique for examining the structure, morphology, and size of extracellular vesicles, including EXOs. By using electron beams, TEM surpasses the resolution limits of conventional optical microscopes, enabling precise visualization of cellular and crystalline structures at the nanoscale. The method operates by transmitting an electron beam through the sample and analyzing the resulting interactions, thereby providing detailed insights into vesicle architecture and composition. It is necessary to note that sample preparation in TEM analysis is highly sensitive and can alter vesicle structure. Additionally, electron beams can damage biological samples in some cases (42, 43).

#### Scanning electron microscopy (SEM)

This method is highly suitable for analyzing nanostructure morphology and identifying chemical compositions. SEM provides imaging at magnifications from 10 $\times$  to 500,000 $\times$  and resolutions below 1–20 nm (sample-dependent). Its ability to examine material surfaces is unparalleled, offering significant advantages over optical microscopes. In SEM, no optical-electronic system forms the image or provides magnification;

instead, images result from point-by-point scanning of the surface via electron-beam interactions with the sample. This yields three-dimensional exosome images (44).

#### Flow cytometry

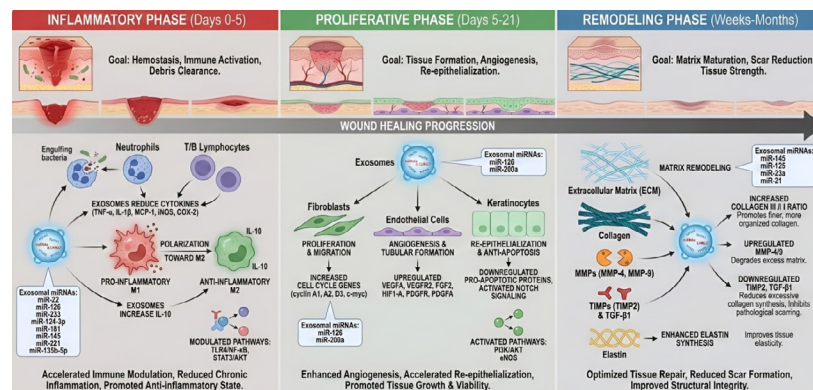
Flow cytometry is a molecular technique for characterizing exosomal surface proteins, enabling multiparametric analysis of marker expression at the single-particle level. Expression levels of multiple exosomal markers, including CD9, CD63, ALIX, TSG101, HSP70, and CD93, can be evaluated by flow cytometry. This technique is well-suited for reproducible analysis of clinical samples, enabling evaluation of the physical and chemical properties of cells, suspended particles in cellular derivatives, and the size or structure of EXOs. However, its detection capacity is limited to particles larger than 300 nm, restricting its utility for smaller vesicles that fall below this threshold (44, 45).

#### Raman spectroscopy

Raman spectroscopy is a vibrational technique based on Raman scattering, widely applied in biology and medicine. The sample is exposed to monochromatic laser light, and photons exchange energy with vibrational states. It measures inelastically scattered photons, producing a Raman spectrum that matches known vibrational modes of specific chemical groups. This enables highly accurate identification of molecular composition and offers valuable insights into the chemical structure of EXOs, including lipid content, membrane protein characteristics, and other surface modifications (46).

#### Stem cell-derived exosomes: emerging therapeutics in wound healing

Stem cell-derived sEVs have been shown to influence all three major phases of wound healing: inflammation, cellular proliferation, and tissue remodeling. During these stages, exosomes modulate key biological processes by either promoting or suppressing specific cellular activities. Experimental studies have demonstrated that sEVs can accelerate tissue repair, regulate granulation tissue formation, enhance epithelialization, and attenuate inflammatory responses. In addition to preclinical evidence, early clinical investigations have also reported promising outcomes. For example, a clinical study conducted in China reported complete wound closure in patients treated with exosome-based therapy within four weeks, compared with 65% healing observed in the control group. Moreover, in burn management, topical exosome formulations such as exosome-based sprays have demonstrated potential to promote skin regeneration and reduce scar formation (Figure 4) (47).



**Figure 4.** Effect of exosome-based therapy on the wound healing process

### *EXOs-therapy in the inflammatory phase: Mechanisms and therapeutic implications*

The inflammatory phase represents the initial and essential stage of wound healing, during which immune responses are activated to remove pathogens, necrotic tissue, and cellular debris, thereby preparing the wound environment for subsequent tissue repair. During this phase, leukocytes are recruited to the injured site to eliminate microorganisms and damaged cells. These immune cells also release cytokines and growth factors that help regulate wound healing. However, excessive or uncontrolled inflammation can impair tissue repair and may contribute to the development of chronic inflammatory conditions. Persistent inflammation has been associated with several pathological conditions, including autoimmune disorders, cardiovascular diseases, type 2 diabetes, neurodegenerative disorders such as Alzheimer's disease, and certain cancers.

Exosomes play a critical role in modulating immune responses by influencing multiple immune cell populations, including macrophages, neutrophils, and both B and T lymphocytes. Their antiinflammatory activity is largely mediated through macrophage polarization toward the antiinflammatory M2 phenotype, inhibition of Tcell proliferation, and suppression of Bcell maturation. These effects collectively reduce the expression of proinflammatory cytokines and enzymes, including TNF $\alpha$ , iNOS, COX2, IL1 $\beta$ , and MCP1. Recent studies have demonstrated that EXOs also increase IL-10 production, an anti-inflammatory cytokine that contributes to improved wound healing and the regulation of scar formation. The immunomodulatory mechanisms of exosomes partially resemble those of mesenchymal stem cells (MSCs). However, while MSCs primarily regulate immune responses through the secretion of cytokines and other soluble factors, exosomes exert their effects primarily by transferring bioactive cargo, including microRNAs (miRNAs), proteins, and lipids, to recipient cells. Exosomal miRNAs have emerged as important regulators of immune modulation and cellular signaling pathways. These small noncoding RNAs influence cytokine expression, immune cell polarization, and intracellular signaling cascades, thereby shaping both innate and adaptive immune responses. For instance, miR22 delivered by bone marrow-derived exosomes promotes macrophage polarization toward the antiinflammatory M2 phenotype, whereas EXO depletion significantly reduces the abundance of M2 macrophages (47,48). Similarly, adiposederived exosomal miR126 has been reported to reduce inflammation and improve tissue damage in myocardial ischemia models. In contrast, exosomal miR233 suppresses the expression of proinflammatory cytokines, including TNF $\alpha$  and IL6. In addition, exosomal miRNAlet7b inhibits the TLR4/NF $\kappa$ B and STAT3/AKT signaling pathways, thereby regulating macrophage activity, promoting M2 polarization, and attenuating inflammatory responses. Other exosomal miRNAs involved in immune regulation include miR1243p, miR181, miR145, miR221, miR126, miR233, and miR135b5p. Furthermore, miR67595p, miR4484, and miR6195p have been reported to inhibit the expression of aging-related genes, including NPM1, PDCD4, and NUP62, in fibroblasts. Collectively, these findings highlight the diverse regulatory roles of exosomal miRNAs in the control of inflammation, immune modulation, and tissue regeneration.

### *EXOs-therapy in the proliferative phase: Enhancing cell*

### *growth and angiogenesis*

Stem cell-derived exosomes significantly influence the proliferative phase of wound healing by enhancing the proliferation and differentiation capacity of cells involved in tissue repair, stimulating angiogenesis, and promoting epithelialization.

EXOs derived from umbilical cord mesenchymal stem cells (UCMSCs) have been shown to accelerate epithelialization and reduce apoptosis in keratinocytes and fibroblasts by down-regulating proapoptotic proteins. At the same time, they promote fibroblast migration and enhance extracellular matrix production (49). MSCderived exosomes also activate the Notch signaling pathway in fibroblasts and endothelial cells, leading to increased expression of extracellular matrix components, including fibronectin, collagen types I and III, and elastin. *In vivo* studies further demonstrated that exometreated wounds exhibit reduced scar formation compared with untreated controls. In addition to these effects, EXO-therapy promotes endothelial cell proliferation and up-regulates several key angiogenic factors, including PDGFR, PDGFA, VEGFA, VEGFR2, FGF2, and HIF1 $\alpha$ . Enhanced cellular proliferation may also be associated with the activation of cellcycle-related genes, including cyclin A1, cyclin A2, cyclin D3, and cMyc.

Moreover, umbilical cord-derived nanovesicles have been reported to reduce cell death by inhibiting the release of apoptosis-inducing factor (AIF) from the nucleus. These vesicles also rejuvenate endothelial cells by up-regulating miR-200a and down-regulating Keap1, thereby mitigating cellular aging and further supporting tissue regeneration (49).

### *EXOs-therapy in epithelialization phase: Matrix remodeling and tissue regeneration*

Numerous studies have demonstrated that exosome therapy promotes angiogenesis both *in vitro* and *in vivo*. For example, Zhang et al. showed that stem cell-derived EXOs significantly enhance the formation of tubular vascular structures in human umbilical vein endothelial cell (HUVEC) models. This proangiogenic effect is mediated by activation of the PI3K/AKT and eNOS signaling pathways and is associated with the transfer of regulatory miRNAs, including miR125a, miR31, miR126, miR130a, and miR319 (44). Recent investigations have also highlighted the role of EXOs in extracellular matrix remodeling and epithelial regeneration. Following exosome therapy, collagen and elastin synthesis are significantly increased, while pathological scar formation is reduced. Umbilical cord-derived exosomal miRNAs—including miR145, miR125, miR23a, and miR21—play an important role in regulating fibroblast differentiation and preventing excessive myofibroblast accumulation. These mechanisms help prevent hypertrophic scar formation. Exosome treatment has also been associated with a 50% increase in type III collagen relative to type I collagen, along with up-regulation of matrix metalloproteinases (MMP4 and MMP9) and down-regulation of the tissue inhibitor TIMP2 (48). In addition, EXOs suppress TGF $\beta$ 1 signaling, thereby reducing fibrotic responses while accelerating epithelialization and overall wound closure.

### *Global regulatory framework for EV-based products*

All EV-based products, including native EVs, EV-drug carriers, and EV-transgene RNA carriers, are classified as "Biological Medicines" (49–51). In the classification of EVs,

their complexity and the active materials they contain are of significant importance. The Committee for Advanced Therapies (CAT) recommends that extracellular vesicles derived from genetically modified cells and containing transgene products be considered “Gene Therapy Products” (52–55). If EVs contain formulated biomaterial components that are primarily responsible for their therapeutic effects, these components are classified as “Class III Medical Devices” (49,56). According to European Union regulations, exosome products are regarded as “Advanced Therapeutic Medicinal Products” (55). In contrast, the US FDA has stricter regulations, classifying exosome products under code 351, as they are still in the “Investigational New Drug (IND)” phase (57,58). The US FDA has warned consumers that, as of March 2025, no exosome products have been approved for therapeutic applications (58).

Among the major regulatory challenges in the field of exosome therapy are several difficulties. Exosomes and other EVs can be secreted by various types of living cells with different physiological functions and therefore cannot be considered a single homogeneous entity (49,50). Moreover, vesicles released from even one specific cell type can be highly heterogeneous in size and in their protein, nucleic acid, and lipid content (59,60). In addition, the cargo and functional properties of EVs may vary depending on the isolation and purification methods used (52). Exosome secretion is also influenced by the conditions under which the source cells are cultured (46). Even when derived from the same parental cell, changes in culture conditions—such as passage number, culture method, medium composition, or atmospheric conditions—can result in exosomes with different physiological characteristics (46, 60–61). These factors lead to the production of distinct products with variable stability across different batches (batch-to-batch variation). Moreover, due to their intrinsic instability, liquid-based exosomes are highly susceptible to degradation in the first hours after production (49,56). These inherent features raise concerns regarding product quality control testing and underscore the need for rigorous regulatory oversight (55, 57).

Furthermore, the development of exosome-based products requires attention to standardizing the administered dose, defining optimal transport and delivery conditions, selecting appropriate routes of administration, and conducting preclinical and early-phase clinical studies (55,57,59). Today, regulatory agencies in various countries are attempting to establish dedicated frameworks and specific regulatory requirements to ensure the safety and efficacy of exosome- and EV-based products (55). For example, the Federal Food, Drug, and Cosmetic Act (FD&C Act) states that compliance with US FDA guidelines and regulations is essential during the manufacturing of exosome-based therapeutic products before they enter clinical use (57,58). The following section discusses some of the most important international regulations and organizations that oversee exosome-based products.

#### *The federal food, drug, and cosmetic act (FD&C)*

According to FD&C regulations, the Office of Cellular, Tissue, and Gene Therapies (OCTGT), located within the FDA's Center for Biologics Evaluation and Research (CBER), is responsible for overseeing cell-based therapies, gene-based therapies, therapeutic cancer vaccines, xenotransplantation products, combination products, and certain devices related to cell and gene therapies (55, 57–58). Given the nature and

function of exosomes, OCTGT is also expected to assume responsibility for regulating exosome-based therapies and cell-derived exosome products (55,57–58). The FD&C further states that before exosomes and exosome-based products can enter clinical use, strict adherence to FDA guidelines and regulatory requirements during the manufacturing of exosome-based therapeutic products is essential (57, 58).

#### *European medicines agency*

The European Directive (2001/83/EC) and Regulation (1394/2007/EC) state that exosomes directly obtained from cells, or those classified based on translated RNA, are not considered Advanced Therapy Medicinal Products (ATMPs) and are instead categorized under biological specifications (55,57). However, if exosomes contain functionally translated RNA and therapeutic outcomes are expected following administration to patients, they may be classified as ATMPs (55, 57).

#### *Ministry of health, labor and welfare (MHLW) and pharmaceuticals and medical devices agency (PMDA) in Japan*

In Japan, regenerative medicine products, pharmaceuticals, and exosome-related products are supervised and regulated by the MHLW and PMDA (57, 58). To obtain marketing approval, MHLW and PMDA carefully review human clinical studies related to each product and issue marketing authorization if the product meets the required standards (57, 58). In Japan, nonliving cell-containing exogenous products are not classified as regenerative medicinal products; instead, they are considered medications under Japanese regulations, because their mechanisms of action primarily rely on pharmacological, immunological, or metabolic processes (57). In contrast, exosome products derived from living cells are classified as biological products and subject to the same legal and regulatory requirements as vaccines, blood products, and other biological therapeutics (55, 57).

In certain cases, upon physicians' request and strictly on an individual basis, noncommercial clinical studies using exosome products may be conducted in Japan. It is important to note that the products used in such studies do not contain viable cells and are not regarded as processed cells (57).

#### *The ministry of food and drug safety (MFDS) of South Korea*

In South Korea, the Ministry of Food and Drug Safety (MFDS) is responsible for regulating and supervising the preparation of medical products (55, 57). The guidelines developed by the Korean Society for Extracellular Vesicles (KSEV) for exosomes closely resemble those issued by the International Society for Extracellular Vesicles (ISEV) (52,55). These guidelines outline quality, nonclinical, and clinical considerations for the development of EV-based products and ensure their safety and efficacy (52, 55). KSEV guidelines have significantly contributed to the rapid growth of exosome applications in aesthetic and cosmetic medicine in South Korea. However, no exosome-based pharmaceutical product has yet received marketing approval in the country (55, 57).

In December 2018, the National Institute of Food and Drug Safety Evaluation (NIFDS) published quality evaluation guidelines and clinical and nonclinical assessment criteria for preparations of extracellular vesicles

intended for the extraction and purification of vesicles secreted by human cells (55, 57).

#### Taiwan food and drug administration (TFDA)

In Taiwan, the Regenerative Medicine Development Act has facilitated the advancement of regenerative medicine for cell derivatives such as exosomes (55, 57). Furthermore, Taiwanese regulatory agencies have referenced guidelines and standards established by various international bodies and organizations, incorporating PIC/S GMP principles and regulations for safety and efficacy testing (55, 57).

In September 2023, Taiwan's Center for Drug Evaluation released a draft guideline for the development and manufacturing control of cellfree vesiclebased medicinal products. This guideline was developed by referencing documents from the International Society for Extracellular Vesicles, Japan's Pharmaceuticals and Medical Devices Agency, and South Korea's Ministry of Food and Drug Safety (52, 55, 57). It encompasses crucial aspects of exosomal product development, including raw material control, preparation, separation, concentration, characterization, quality control, stability, and integrity (52, 55).

#### Iranian food and drug administration

The demand for services related to cellular products and their derivatives, particularly extracellular vesicles, has surged globally and in Iran. Driven by this increasing need and escalating demand, Iran's healthcare system must transition into a new phase of translating knowledge in this field from research to technology, culminating in the introduction of products and, ultimately, the provision of clinical services aligned with the therapeutic market's needs (55, 57).

Adhering to the rules and requirements for manufacturing EVbased pharmaceutical products, as well as complying with the regulations governing their distribution and consumption within the pharmaceutical system, is essential (55, 57). The IFDA, committed to safeguarding patients and ensuring compliance with international laws, considers advancing an efficient pathway for the safe and effective development of novel therapies using cell derivatives—especially EVs—as one of its core missions (21, 55, 57). In this regard, to facilitate

potential approvals, a comprehensive guideline, similar to those for other cell, tissue, and gene therapy products, has been proposed for cell derivative and extracellular vesicle products. This guideline covers the preparation and submission of the product's technical dossier to the Iranian Food and Drug Administration; obtaining approvals for safety, quality, and efficacy before market launch; securing an IRCT code; and registering the product in the TTAC system (55, 57). A summary of the related process is depicted in the accompanying document. (Figure 5).

#### Exosome administration

Exosome administration in clinical studies has been investigated via multiple routes, including inhalation, topical application, intraarticular injection, intracerebral or spinal injection, and systemic administration. These approaches aim to improve therapeutic efficacy, enhance delivery to target tissues, and reduce rapid clearance of EXOs from the circulation.

Although standardized dosing protocols for exosome therapy have not yet been established, most studies report using 50–500 µg of exosomal protein per milliliter of the injection, depending on the disease indication, route of administration, and therapeutic objectives (49).

Recently, the Iranian Food and Drug Administration categorized clinical applications of extracellular vesicle (EV)–based products into several major domains:

#### Topical products based on EV/exosome derivatives

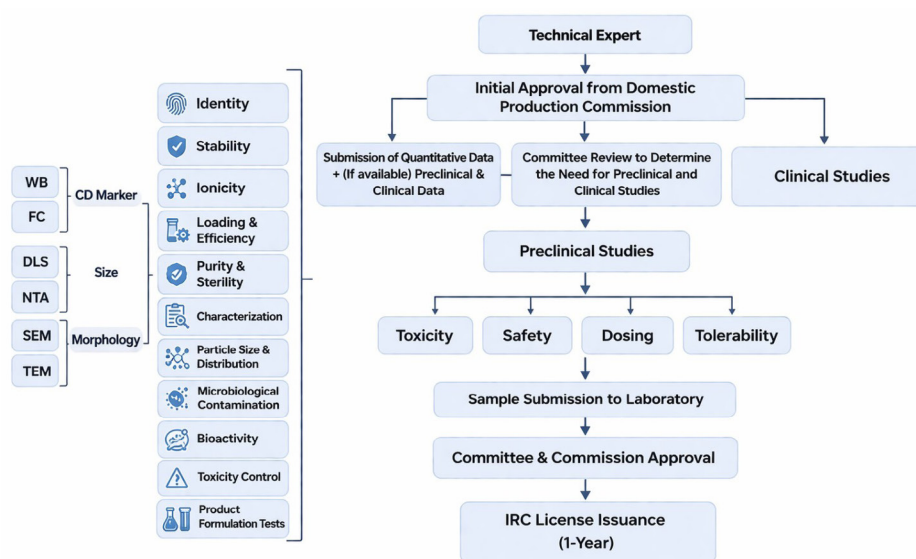
These products are typically derived from plant sources or noncellular materials and are commonly used in cosmetic or mesotherapy applications through topical administration.

#### Cosmeceutical, regenerative, therapeutic products

This category includes exosomebased cosmetic, restorative, and therapeutic formulations designed for aesthetic or dermatological applications. These products are generally intended for topical use and are not administered by injection.

#### Systemic administration

These products are derived from live cells with normal



**Figure 5.** The regulatory pathway for the development and clinical implementation of sEV-based products, according to the IFDA regulatory guideline

physiological function and are intended for systemic therapeutic applications. Approximately 55% of clinical trials involving EV-based therapies utilize systemic administration.

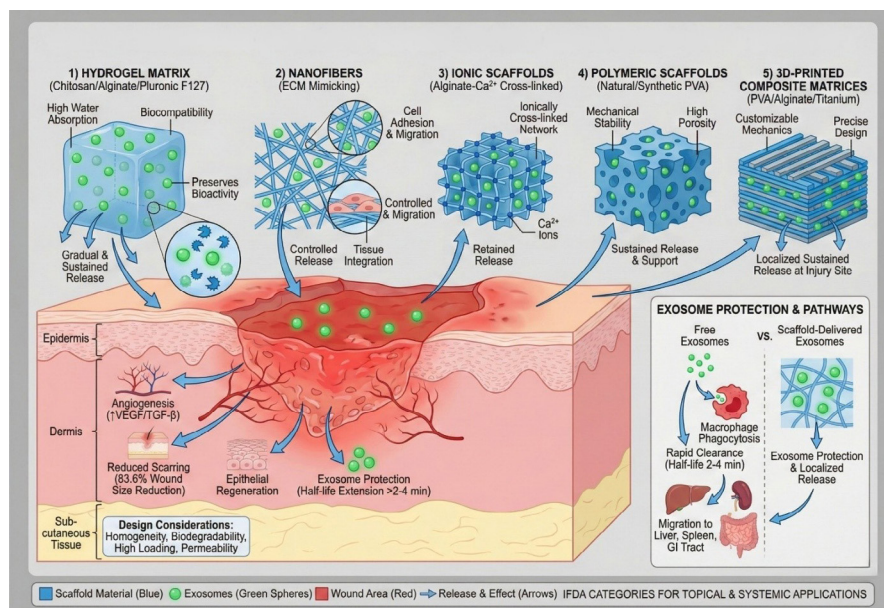
#### *Advanced scaffolds for exosome-based therapy in tissue regeneration*

Beyond IFDA classifications, advanced smart delivery platforms, including hydrogel matrices, nanofibers, ionic scaffolds, polymeric scaffolds (natural and synthetic), and 3D composite matrices, have been developed to overcome challenges in exosome therapy, particularly rapid clearance from the bloodstream or injection site. Following administration, EXOs quickly redistribute to the liver, spleen, lungs, and gastrointestinal tract, where they undergo degradation/elimination, primarily through macrophage-mediated phagocytosis in the liver and spleen. sEVs exhibit an extremely short plasma half-life (2–4 minutes); however, macrophage inhibition can markedly prolong their circulation time. To enhance stability and therapeutic lifespan, bio-scaffolds and exosome matrices are employed to provide protection, retention, and controlled release, thereby preventing premature degradation while preserving exosome structure and function at the injury site. These systems improve therapeutic efficacy by facilitating host-cell interactions and stimulating tissue repair. Exosome-loaded scaffolds must preserve their structural integrity, physicochemical stability, biological functionality, and morphological characteristics to ensure effective and sustained retention within the target tissue microenvironment. By maintaining these properties, scaffolds can provide a controlled release of EXOs, prolong therapeutic activity, and support regenerative processes, including angiogenesis, epithelialization, and extracellular matrix remodeling. Moreover, effective scaffold design must ensure long-term retention within the target environment, protection of EXOs from immune or environmental degradation, and a gradual, sustained release profile to maximize therapeutic efficacy. Effective scaffold integration with damaged tissue and timely nanoparticle transfer are critical for efficacy. Design considerations for smart controlled-release systems include uniform exosome distribution (homogeneity), biocompatibility, biodegradability, and mechanical stability (particularly in high-pressure tissues such as skin and muscle), physiological stability, targeted release kinetics, high permeability, strong host cell interaction (adhesion, proliferation, and differentiation), and high loading capacity. Key scaffolds for controlled exosome release include hydrogels, nanofibers, ionic and polymeric (natural or synthetic) scaffolds, and three-dimensional composite matrices. Hydrogels provide 3D structure, high water absorption, excellent biocompatibility, and the ability to preserve physicochemical properties under diverse conditions, ideal for loading and gradual release (59). They provide a moist wound environment, protect EXOs from enzymatic degradation, and enable gradual, controlled release at the wound site through tunable pore size, cross-link density, and viscosity, enhancing stability and therapeutic efficacy, thereby preserving bioactivity, increasing bioavailability, and stimulating repair processes, such as angiogenesis, epithelial regeneration, and collagen synthesis. Hydrogel-host tissue integration facilitates peripheral cell migration and effective exosome interaction,

accelerating regeneration. Experimental evidence highlights that chitosan hydrogels loaded with EXOs reduce wound size by 83.6%, achieve complete epithelial regeneration, and enhance keratinocyte migration. Alginate hydrogel with exosomes enhances type I/III collagen synthesis by 40%, increases vascular density, and halves healing time up to 50% (60). Pluronic F127 hydrogel preserves exosome activity for up to 14 days, prevents proteolytic degradation, and triples bioavailability. However, umbilical cord-derived EXOs combined with Pluronic F127 accelerate diabetic wound closure, elevate VEGF/TGF- $\beta$ , reduce granulation, enhance angiogenesis, and achieve full skin regeneration. Chitosan/silk hydrogel loaded with gingival MSC EXOs improves collagen organization, reduces fibrosis, enhances angiogenesis, and increases vascular density (61).

Beyond hydrogels, advanced scaffolds such as ionic, nanofibrous, and photopolymer scaffolds mimic the extracellular matrix, creating environments that support cell adhesion, migration, and uniform exosome distribution. Among these, ionic cross-linked alginate scaffolds are widely used for exosome retention and controlled release (41). Alginate, a natural polysaccharide derived from brown algae and marine species, consists of mannuronic and guluronic acid monomers. When combined with divalent ions such as calcium, it forms a three-dimensional egg-crate-like cross-linked network. Its biocompatibility, biodegradability, non-toxicity, and high-water absorption make alginate particularly suitable for exosome loading and gradual release. Shafei et al. designed an alginate scaffold combined with EXOs for skin wound repair, thereby accelerating the wound-healing rate, enhancing collagen synthesis, and improving angiogenesis compared to pure alginate. Another study showed that umbilical cord MSC-derived exosome–alginate constructs achieved successful outcomes in neural damage repair (48).

Photopolymer scaffolds, formed through polymerization under UV or visible light in the presence of photoinitiators, represent versatile platforms for exosome delivery. Commonly used materials include chitosan (a natural, biocompatible, biodegradable polymer with strong tissue adhesion) and gelatin (a denatured collagen derivative with high cell adhesion and biodegradability), both of which are ideal for optical scaffold applications. One study has developed an injectable photopolymer chitosan-methacrylate hydrogel for human MSC-derived exosome delivery, demonstrating remarkable cranial defect bone repair in murine models (62). Similarly, gelatin methacrylate has emerged as a tunable optical scaffold with high regenerative potential, offering controlled mechanical properties and enhanced suitability for tissue engineering and regenerative medicine. Three-dimensional-printed scaffolds offer precise design and selective loading of cells, biomaterials, and growth factors, with customizable properties tailored to the mechanics and biology of target tissues. Typically fabricated from biocompatible natural or synthetic polymers, ceramics, or composites, these scaffolds have broad applications in tissue engineering, regenerative medicine, and drug delivery. Encapsulation of EXOs within 3D scaffolds enables their gradual, sustained release at the injury site, thereby enhancing therapeutic efficacy. For example, polyvinyl alcohol/alginate composites have been applied in diabetic wound repair, while titanium-based 3D scaffolds loaded with EXOs promote mesenchymal stem cell osteogenic differentiation and support cell-free



**Figure 6.** Advanced scaffolds for exosome delivery in wound repair

bone regeneration. By stimulating bone-related signaling pathways and activating natural tissue cells through paracrine mechanisms, 3D bio-nanovesicle scaffolds demonstrate significant potential for hard tissue repair and advanced regenerative therapies.

#### **Next-generation strategy for integrating sEVs with therapeutic agents in advanced drug delivery systems**

In recent decades, extracellular vesicles, particularly sEVs, have demonstrated diagnostic and therapeutic applications due to their biological complexity, including gene therapy, early disease detection (through biomarkers), immune activation (via vaccines), drug delivery (as carriers), and miRNA-based treatment for neurological diseases (49–51). However, it is important to note that natural exosomes (sEVs released from various cells) generally have a limited half-life in clinical applications (49, 50). Additionally, their parent cell-defined molecular composition naturally lacks specific engineered therapeutic cargo, creating inherent limitations for clinical use (50, 51).

To overcome these challenges, natural sEVs can be engineered and structurally modified using biotechnology and nanotechnology according to specific therapeutic or diagnostic requirements, yielding so-called engineered exosomes (49–51). Engineered exosomes, nanoscale sEVs deliberately modified to enhance their biological performance, have emerged as promising biotherapeutic agents due to their complex bioactive components and diverse pharmacological properties (49–51). The ability to selectively load drugs, therapeutic RNAs (e.g., siRNA, miRNA), monoclonal antibodies, and immunomodulatory molecules into engineered exosomes has transformed these smart, targeted nanovesicles into highly effective tools for both therapeutic and diagnostic purposes (49–51). These features open new horizons in advanced therapies, including personalized medicine, immunotherapy, neurological and inflammatory treatment, and the development of intelligent nanomedicine delivery systems (49–51).

Numerous studies have demonstrated that engineered sEVs can enhance the therapeutic effects of drugs and chemical compounds. Recently, engineered sEVs loaded

with miR140 have been designed to specifically deliver microRNA to chondrocytes, offering a potential therapeutic approach for osteoarthritis (49–51). Recent studies highlight the effects of engineered sEVs in treating cancer, diabetes, cardiovascular diseases, and osteoarthritis through anti-inflammatory and antioxidant mechanisms, rescuing tissue damage in injury models, and suppressing inflammatory activation to support tissue repair (52). From 2022 to 2025, innovations in surface modification and cargo loading have strengthened precision drug delivery, driving progress in combating cancer drug resistance and regenerating diverse tissues, including bone, cartilage, liver, nerve, kidney, and skin (53–55).

Despite their many benefits and medical applications, engineered exosome therapy faces various challenges, including method-dependent inconsistencies, potential pathogen contamination (e.g., bacteria, viruses), variable composition, loss of therapeutic components during processing, ethical and regulatory concerns regarding tissue sourcing and cultural or religious beliefs, time-consuming and costly procedures, data privacy issues, lack of standardization and regulation, and limited scientific validation (49, 55, 56). Regulatory bodies such as the FDA and EMA are developing guidelines for characterization, safety, and efficacy, emphasizing GMP manufacturing, confirmation of mechanism of action (MOA), and clinical trials to address immunogenicity and longterm effects (55–57).

Future directions include scalable production, single-vesicle analytics, humanized models, and stimulus-responsive designs to overcome heterogeneity and enhance efficacy (56–64).

#### **Conclusion**

To date, exosome therapy has been employed to treat various diseases, including dysplasia, COVID-19, bronchopneumonia, type 1 diabetes, macular degeneration, and neurological conditions such as stroke and acute ischemia, with promising outcomes. In the field of regenerative medicine and tissue engineering, stem cell-derived EXOs have been widely investigated in preclinical studies for skin wound repair across animal models,

including mice, rats, rabbits, and dogs. These studies consistently demonstrate the remarkable efficacy of exosome therapy in accelerating wound healing, enhancing angiogenesis, improving epithelialization, and reducing wound closure time and scar formation. However, structural and physicochemical differences between human skin and that of the aforementioned laboratory animals highlight the need for validation in models more closely resembling human physiology. Porcine skin, which closely mirrors human skin in structure, thickness, extracellular matrix composition, and physiological characteristics, is a valuable model for advancing translational research. Therefore, evaluating exosomal biofactors in porcine wound models may be a crucial step toward translating this technology for clinical use in humans. Although the therapeutic potential of exosome therapy in animal models is highly encouraging, its clinical applications in humans remain limited.

One of the most important aspects of EVs and EV-based therapy is the quantitative and qualitative characteristics of EV products. Following quality approval, the safety and efficacy of these products must be confirmed through various preclinical and clinical trials. All processes must be controlled and monitored in accordance with the standard guidelines or protocols approved by the relevant regulatory agencies. The global regulatory frameworks establish stringent manufacturing standards to guarantee the consistent quality and sterility of EVs products, making product release licenses contingent upon meeting these requirements. Even post-approval, regulatory agencies continuously monitor product performance to detect any unforeseen safety issues or adverse events. In Iran, the IFDA classifies these products based on their risk and intended use, which dictates the level of scrutiny and the specific evidence required for market authorization. Currently, this regulatory framework provides a clearer translational roadmap for the development and clinical implementation of sEV-based therapies. However, the IFDA guideline has several limitations, including Lack of detailed clinical trial protocols, inadequate coverage of therapeutic application, incomplete alignment with the global regulatory frameworks, Limited approval experience for EV-based products, absence of specific protocols for large-scale production, and insufficient long-term safety assessment parameters, particularly concerning immunogenicity, tumorigenicity, and other potential adverse effects.

### Acknowledgment

We are grateful to the Yara Institute for supporting this research.

### Ethical Considerations

This study is a narrative literature review and does not involve the use of primary data from human subjects or animal experimentation. Therefore, formal ethical approval and informed consent were not required.

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Data Availability Statement

No new data were created or analyzed in this study. Data

sharing does not apply to this article.

### Authors' Contribution

B H and D N conceptualized and designed the review. D N, N A, and Z S conducted the literature search, screened the literature, and collected the relevant information. D N prepared the original draft. N A designed and prepared the figures using Adobe Illustrator. D N, B H, N A, and Z S reviewed and edited the manuscript. B H supervised the work. All authors read and approved the final version of the manuscript.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Declaration

The authors declare that they have not used AI-generated work in this manuscript.

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