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Mesenchymal stromal cell–derived extracellular vesicles in regenerative medicine: Standardisation, bioengineering and clinical translation

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ABSTRACT

Mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) have emerged as promising cell-free therapeutics that recapitulate key paracrine functions of MSCs whilst mitigating limitations of viable-cell therapies. Clinical translation is hindered by inconsistent dose metrics, lack of validated potency assays, and manufacturing complexities. Here we review advances in MSC-EV standardisation, source and culture determinants, engineering and delivery platforms, and clinical applications, exemplified by chronic wound healing. We operationalise dual-metric dosing (particles *plus* protein) linked to mechanism-aligned potency assays, adopt a route-aware exposure–response framework to guide delivery strategies, and propose decision matrices aligning MSC sources and bioprocesses with indication-specific tasks. Good Laboratory Practice safety panels and Good Manufacturing Practice scale-up strategies are outlined to support regulatory readiness. Early clinical studies demonstrate feasibility and short-term safety but reveal heterogeneous efficacy, underscoring the need for harmonised dosing and potency measures. Collectively, these insights provide a roadmap to advance MSC-EVs as indication-matched regenerative medicines.

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; ABC, accelerated blood clearance; AD-MSC, adipose-derived MSC; AE, adverse event; ALP, alkaline phosphatase; α-SMA, alpha-smooth muscle actin; Ang1, angiopoietin-1; ARDS, acute respiratory distress syndrome; ARG1, arginase-1; BBB, blood–brain barrier; BD, biodistribution; BDNF, brain-derived neurotrophic factor; BM-MSC, bone marrow MSC; CDMO, contract development and manufacturing organisation; CFSE, carboxyfluorescein succinimidyl ester; CMC, chemistry manufacturing and controls; CQA, critical quality attribute; DC, dendritic cell; dECM, decellularised extracellular matrix; DP-MSC, dental pulp MSC; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; EV, extracellular vesicle; F127, Pluronic F127; Gal8, galectin-8; GelMA, gelatin methacryloyl; GFR, glomerular filtration rate; GLP, Good Laboratory Practice; GMP, Good Manufacturing Practice; GvHD, graft-versus-host disease; HFB, hollow-fibre bioreactor; HGF, hepatocyte growth factor; HLA-G, human leucocyte antigen G; HSC, hepatic stellate cell; HSPG, heparan sulphate proteoglycan; HUVEC, human umbilical vein endothelial cell; IA, intra-articular; IDO, indoleamine 2,3-dioxygenase; IL-1RA, interleukin-1 receptor antagonist; iMSC, induced pluripotent stem cell-derived MSC; IN, intranasal; ISEV/ISCT, International Society for Extracellular Vesicles/International Society for Cell & Gene Therapy; IV, intravenous; LNP, lipid nanoparticle; LVEF, left ventricular ejection fraction; lyo, lyophilisation; MI, myocardial infarction; MISEV, Minimal Information for Studies of Extracellular Vesicles; MMP, matrix metalloproteinase; MOA, mechanism of action; MPS, mononuclear phagocyte system; MSC-EV(s), mesenchymal stromal cell-derived extracellular vesicle(s); NETosis, neutrophil extracellular trap formation; NHP, non-human primate; NR, not reported; PCL, polycaprolactone; PD, pharmacodynamics; PD-L1, programmed death-ligand 1; PEG, polyethylene glycol; PGE_2, prostaglandin E_2; PI3K, phosphoinositide 3-kinase; PK, pharmacokinetics; PVA, poly(vinyl alcohol); QbD, Quality by Design; QC, quality control; RCT, randomised controlled trial; RGD, arginine-glycine-aspartic acid peptide; ROS, reactive oxygen species; RVG, rabies virus glycoprotein peptide; SEC, size-exclusion chromatography; sEV, small extracellular vesicle; SMAD, mothers against decapentaplegic homolog proteins; SOC, standard of care; SOP, standard operating procedure; SPION, superparamagnetic iron oxide nanoparticle; TAT, thrombin–antithrombin; TBI, traumatic brain injury; TF, tissue factor; TFF, tangential-flow filtration; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; Treg, regulatory T cell; TSG-6, tumour necrosis factor-stimulated gene 6; UC-MSC, umbilical cord MSC; VAS, visual analogue scale; VEGF, vascular endothelial growth factor; VFDs, ventilator-free days; Wnt4, Wnt family member 4; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

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1. Introduction

Cell-based therapies, particularly those using mesenchymal stromal cells (MSCs), have long been explored for their potential to restore tissue function in a range of degenerative and injury-related conditions. Whereas early work focused on multilineage differentiation and engraftment [1], it is now widely recognised that the principal therapeutic benefits of MSCs arise from paracrine signalling rather than direct tissue replacement [2]. Among the secreted factors mediating these effects, extracellular vesicles (EVs) have emerged as pivotal messengers, driving a shift towards cell-free regenerative approaches [3].

MSCs are defined by adherence to plastic in culture, a characteristic surface-marker profile, and multipotent differentiation potential [4]. They exhibit broad immunomodulatory, pro-angiogenic, and trophic activities and can be isolated from multiple tissues (bone marrow, adipose, and perinatal sources) [1,2,5,6]. Yet clinical translation of MSC-based cell therapies has been constrained by inter- and intra-donor heterogeneity, limited proliferative capacity in vitro, and safety concerns (e.g., immunogenicity, tumourigenicity) [7,8], alongside the logistical and cost burdens of manufacturing, cryopreserving, and distributing viable products at scale [9].

MSC-derived extracellular vesicles (MSC-EVs) have gained traction as an alternative modality that reproduces many of the beneficial effects of MSCs whilst overcoming several limitations associated with live-cell therapies [10]. These nanoscale, lipid-bilayer vesicles carry proteins, lipids and nucleic acids (including mRNAs and microRNAs) that can modulate immune responses, stimulate angiogenesis and regulate fibrotic and apoptotic pathways [11,12]. In preclinical models, MSC-EVs have been shown to enhance wound healing and protect against organ injury in cardiovascular, neurological and musculoskeletal settings [13–15]. Notably, MSC-EVs are non-replicative—that is, they cannot divide or form new cells—and are generally less immunogenic than intact cells, while being amenable to Good Manufacturing Practice (GMP)-aligned bioprocesses [16,17]. For example, in cutaneous repair, advances in bioengineering and rigorous safety testing are beginning to address key translational hurdles [18].

Despite these promising developments, the clinical translation of MSC-EVs remains constrained by several persistent barriers, including the absence of validated potency assays, inconsistent dose metrics (such as particles, protein content, or 'cell equivalents'), limited exposure–response data, and substantial heterogeneity in EV isolation and characterisation methods [19,20]. In addition, evolving regulatory frameworks for EV-based products create uncertainty around classification and approval pathways [21]. Meanwhile, there is a growing demand for safe, distributable regenerative therapies for conditions such as chronic wounds, osteoarthritis, neurodegenerative disorders, and inflammatory diseases [22–24]. For example, Shimizu et al. [18] demonstrated that indication-specific optimisation of MSC-EVs, together with rigorous standardisation and safety testing, can serve as a practical model for advancing the field.

This narrative review synthesises advances and translational challenges in MSC-EV therapeutics across four key domains: (i) standardisation and quality control (QC), (ii) source and culture determinants, (iii) engineering and delivery platforms, and (iv) clinical applications and translation, with chronic wound healing as a worked example. In addition to summarising the field, we: (1) introduce dual-metric dose reporting (particles plus protein) linked to mechanism-of-action (MOA)-aligned potency assays; (2) apply a route-aware exposure–response framework to guide delivery and depot design; (3) present a practical decision matrix that aligns MSC source, conditioning and process with indication-specific biological tasks; (4) outline Good Laboratory Practice (GLP) safety testing (e.g., thrombin generation and complement activation) and comparability triggers for process changes; and (5) map Good Manufacturing Practice (GMP)-aligned scale-up strategies (e.g., tangential-flow filtration (TFF), hollow-fibre bioreactors (HFB)) to critical quality attributes (CQAs), release testing and stability. Together, these elements provide a translational roadmap intended to convert MSC-EVs from promising research tools into regulator-ready, indication-matched therapeutics.

2. Methodology

This narrative review synthesises current knowledge on MSC-EVs in regenerative medicine using a broad-based literature survey, rather than a strict systematic approach, consistent with best practice for narrative reviews. To enhance transparency, we pre-specified information sources, search strings, eligibility criteria, and screening/extraction steps as outlined below.

2.1. Information sources and date limits

A comprehensive search was performed in PubMed/MEDLINE, Web of Science, and Scopus for publications from January 1, 2008 to August 31, 2025. Reference lists of key reviews, consensus guidelines (MISEV2018; MISEV2023) and position papers were hand-searched to identify additional primary sources [25,26]. ClinicalTrials.gov and trial publications were consulted to capture registered or completed clinical studies relevant to MSC-EVs.

2.2. Core search strategies

Database-tailored Boolean strategies combined concepts for MSCs, EVs, regenerative indications, and engineering/bioprocess topics. A representative PubMed string was:

("mesenchymal stromal" OR "mesenchymal stem" OR MSC) AND ("extracellular vesicle*" OR exosome* OR microvesicle* OR "small extracellular vesicle*") AND (regeneration OR "wound healing" OR fibrosis OR osteoarthritis OR stroke OR "spinal cord injury" OR "myocardial infarction") AND (bioengineering OR delivery OR hydrogel OR scaffold OR PEGylat* OR "tangential-flow filtration" OR ultracentrifugation OR "size-exclusion chromatography" OR potency OR "quality control" OR "Good Manufacturing Practice").

Equivalent term sets and field tags were adapted for Web of Science and Scopus. "MISEV", "ISEV", and "potency assay" were included to retrieve standards and assay-development literature.

2.3. Eligibility criteria

Inclusion: Peer-reviewed original research, clinical studies, and reviews/guidelines addressing MSC-EV biology, engineering/delivery, manufacturing/QC, safety, dosing/pharmacology, or clinical translation; human, animal, and in vitro mechanistic studies; English-language publications.

Exclusion: Studies without an EV fraction (e.g., unfractionated conditioned medium), non-MSC EVs unless directly comparative, oncology-only applications without regenerative relevance, conference abstracts, editorials and letters without primary data, and non-English items. Preprints (2024–2025) were cited sparingly and labelled as such when they contributed unique, timely evidence.

2.4. Study selection and data extraction

Titles/abstracts were screened in duplicate by at least two authors; full texts were then reviewed for eligibility, with disagreements resolved by discussion involving a third author. Duplicates were removed using reference-management tools and manual checks. From each eligible item, we extracted: MSC source and donor attributes; culture/priming conditions; EV isolation/purification method(s); characterisation against MISEV-recommended markers; dose metric(s) and amount; route and delivery platform; model/indication; mechanistic readouts (e.g., immunomodulation, angiogenesis, anti-fibrosis); safety signals; and, where applicable, clinical endpoints and assessment windows. For

Table 1
Regenerative mechanisms of MSC-derived EVs.

Mechanism	Representative MSC-EV cargo	Primary effects	Suggested potency assay (lot release/ development)	Key references
Immunomodulation	miR-21-5p, miR-146a, let-7 family, PGE_2, PD-L1, IDO, HLA-G, annexin A1	Macrophage M1→M2 transition; tolerogenic DCs; T-cell suppression/Treg induction; reduction of NETosis	Macrophage polarisation assay (IL-6/IL-10 ratio; ARG1/CD206 flow cytometry); CFSE-based CD3/CD28 T-cell proliferation suppression; DC maturation inhibition (CD80/CD86↓, IL-12↓)	[39–42, 52]
Angiogenesis & vascular regeneration	VEGF, Ang1, HGF, Wnt4, miR-126, miR-132, miR-210	Endothelial proliferation/migration; tube formation; β-catenin activation; PI3K/AKT/eNOS signalling	HUVEC tube-formation (branch points/length); endothelial scratch migration; nitric-oxide bioactivity (DAF-2 DA); aortic-ring sprouting (development)	[43–45]
Cytoprotection/anti-apoptotic effects	Bcl-2, survivin, HSPs, miR-19a, miR-21, miR-24, miR-182	Inhibition of BAX/caspase pathways; cytoprotection after ischaemia/oxidative stress	Stress-challenge viability (e.g., H_2O_2 or cytokine mix); caspase-3/7 activity; mitochondrial membrane potential (JC-1/TMRM)	[40,42]
Anti-fibrotic activity	miR-29 family, let-7 family, miR-148a, TIMPs; anti-TGF-β/SMAD cargo	Blockade of myofibroblast transdifferentiation; reduced α-SMA expression and collagen deposition; restoration of ECM turnover	TGF-β-stimulated fibroblast assays: α-SMA (IF/flow) and COL1A1/COL3A1 (qPCR/ELISA); pSMAD2/3 Western blot; collagen gel contraction (development)	[46–48]
Direct cellular reprogramming	Functional mRNAs, transcription factors, miR-124, BDNF	Gene-expression remodelling; neurotrophic and angiogenic programme activation	Neurite-outgrowth assay (e.g., primary neurons/PC12); target-gene modulation (qPCR/protein) linked to intended MOA	[40,42]
Microenvironmental modulation	Pro-resolving mediators (e.g., annexin A1), ECM-remodelling enzymes (MMPs/TIMPs), mitochondrial components	Immune-niche re-education; ECM normalisation; resolution of inflammation	MMP/TIMP activity assays; NETosis suppression (MPO-DNA complexes); macrophage efferocytosis index (development)	[49,50]
Cargo delivery & uptake	CD63, CD81, integrins (e.g., αvβ3), HSPG interactions; targeting ligands (e.g., RVG)	Target-cell recognition; endocytosis; cytosolic cargo release	Cellular-uptake kinetics (pH-sensitive dyes); receptor/ligand blockade (e.g., heparin/HSPG competition); Gal8-recruitment endosomal-escape assay (development)	[28,51]

Ang1, angiopoietin-1; BDNF, brain-derived neurotrophic factor; DC, dendritic cell; ECM, extracellular matrix; eNOS, endothelial nitric-oxide synthase; GvHD, graft-versus-host disease; HGF, hepatocyte growth factor; HLA-G, human leucocyte antigen-G; HSPG, heparan sulphate proteoglycan; IDO, indoleamine-2,3-dioxygenase; IL-1RA, interleukin-1 receptor antagonist; MMP, matrix metalloproteinase; NETosis, neutrophil extracellular trap formation; PD-L1, programmed death-ligand-1; PI3K, phosphoinositide-3-kinase; PGE_2, prostaglandin E_2; TIMP, tissue inhibitor of metalloproteinases; Treg, regulatory T cell; α-SMA, alpha-smooth muscle actin; VEGF, vascular endothelial growth factor; Wnt4, Wnt family member 4. (Assay abbreviations used above: ARG1, arginase-1; CFSE, carboxyfluorescein succinimidyl ester; Gal8, galectin-8.)

engineering/manufacturing papers, we additionally captured loading strategy, targeting motif(s), scalability platform (e.g., TFF, HFB), and comparability/potency data [25,26].

2.5. Synthesis approach and presentation

Given the heterogeneity of study designs, we used a narrative, thematic synthesis organised to mirror the manuscript structure (standardisation/quality; source and culture determinants; engineering/delivery; clinical applications and translation). Tables consolidate mechanisms (Table 1), platforms (Table 2), and representative preclinical/clinical data with an emphasis on dosing units and assessment windows (Table 3), whilst Table 4 summarises translational gaps and enabling solutions.

Reporting conventions: Throughout, we use EV/sEV terminology in line with MISEV2023, reserving "exosome" only when endosomal origin is demonstrated [14,26].

As this is a narrative review rather than a systematic review or meta-analysis, certain limitations apply. Study selection may be influenced by selection and publication bias, and the restriction to English-language literature may exclude relevant non-English studies. Formal risk-of-bias assessment and quantitative synthesis were not performed due to methodological heterogeneity. Recent references (2024–2025) were checked for bibliographic details and peer-review status, with preprints explicitly identified. Statements about efficacy are qualified by study design and cross-referenced with guideline papers where possible [25,26].

3. Fundamentals of MSC-derived EVs

MSC-EVs are heterogeneous, nanoscale, lipid-bilayer particles that mediate intercellular communication and partially reflect the phenotype and physiological state of their parent MSCs. Understanding the classification, biogenesis, and cargo composition of MSC-EVs is essential for interpreting their regenerative potential and designing reproducible preclinical and translational studies [25,26].

3.1. Biogenesis and classification of EVs

EVs are commonly grouped into three subtypes according to their biogenesis and typical size, recognising that these categories overlap and are method-dependent [25].

- Exosomes (≈30–150 nm): Derived from the endosomal system. Intraluminal vesicles (ILVs) form within multivesicular bodies (MVBs) by inward budding, and are released after MVB-plasma-membrane fusion. This process involves endosomal sorting complexes (ESCRT) complexes (0-III), accessory proteins such as Tumour susceptibility gene 101 (TSG10) and ALG 2-interacting protein X (ALIX), Rab GTPases, and ESCRT-independent routes involving ceramides and tetraspanins [27].
- Microvesicles (MVs) (≈100–1000 nm): Produced by outward budding of the plasma membrane. This process is regulated by small GTPases (ARF6, Rab, RhoA-ROCK), phospholipase D, protein kinase C and calcium-dependent calpain activation, with ESCRT 0-III and Vps4 complex contributing to final scission [28].
- Apoptotic bodies (≈500–5000 nm): Released from apoptotic cells, these vesicles contain nuclear fragments, organelles and cytoplasmic material. Once regarded as inert debris, they are now recognised as context-dependent bioactive particles capable of eliciting anti-inflammatory or tolerogenic responses [26].

Because markers and size ranges overlap, ISEV recommends using operational terms such as "small EVs" (<200 nm) and "medium/large EVs" (>200 nm) when the biogenetic origin is uncertain. ISEV also recommends reporting the separation and quantification methods, the

Table 2
Engineering and delivery platforms for MSC-EV therapeutics.

Platform/Strategy	Representative methods/ materials	Primary objectives	Exemplar indication/ readouts	Limitations/risks	Key references
Delivery strategies					
Intravenous (IV)	Peripheral IV bolus/infusion; labelled EVs for PK-BD	Rapid systemic exposure; whole-body distribution profiling	Rodent/NHP PK-BD; organ accumulation; exposure-response relationships	Rapid MPS clearance; off-target hepatic/splenic uptake; potential complement/coagulation activation with IV dosing; repeat-dose accelerated clearance; dosing heterogeneity	[62,63]
Local injection (intramuscular/ intra-articular/ intramyocardial)	Perilesional or target-organ injection; image-guided delivery	↑ Local exposure and residence; reduced systemic dilution	Joint/soft-tissue models: retention, dispersion radius; pain/function endpoints (pre-specified windows)	Diffusion/lymphatic clearance; injection-site variability; may require repeat dosing; dose normalisation across tissue volumes	[62,64]
Intranasal (nose-to-brain)	IN drops/atomiser; olfactory/ trigeminal pathway targeting	BBB bypass; CNS exposure	Brain uptake (%ID/g by region); neurobehavioural recovery; inflammatory biomarkers	Deposition variability; mucociliary clearance; pulmonary spill-over; dose metering	[65]
Inhalation/nebulised	Mesh/jet nebulisers; aerosol integrity characterisation	Targeted pulmonary delivery; reduced systemic exposure	Inhaled/retained fraction; regional lung deposition; distal-organ targeting (where relevant)	Aerosol shear damage; device compatibility/standardisation; dose-per-breath normalisation	[66]
Topical/ophthalmic	Sprays, gels, ophthalmic drops, microneedles	Non-invasive local delivery; surface repair	Corneal injury: re-epithelialisation, ↓ inflammation; dermal repair readouts	Short residence (tear/skin turnover); preservative sensitivity; ocular surface immunoreactivity; dosing-frequency constraints	[67,68]
Depot - Hydrogel	GelMA, alginate, fibrin, PVA hydrogels (including oxygen-releasing/self-healing variants)	Retention; sustained release; cargo protection	Full-thickness wound models: closure %, angiogenesis, M1→M2 shift	Loading uniformity; burst on swelling; matrix-cargo interactions altering EV phenotype; sterilisation/scale-up challenges	[77,83]
Depot - Scaffold	Natural scaffolds (collagen, dECM); synthetic (PCL, ceramics; 3D-printed)	Structural support; localised EV anchoring; osteo/angiogenesis	Bone defect: μCT bone volume; tensile strength; vessel density	Mechanical mismatch; diffusion limits; inflammatory response; sterilisation and batch variability	[89]
EV engineering strategies					
Surface modification and targeting	PEGylation; Lamp2b-RVG; cyclic RGD tags; platelet-mimetic cloaking; SPION loading/ magnetic guidance	Immune evasion; tissue-specific uptake; enhanced localisation	Target-organ EV accumulation; functional improvement at lesion site	Anti-PEG antibodies/accelerated blood clearance; ligand immunogenicity; SPION dose limits; altered PK on repeat dosing; added CMC complexity	[63–72]
Cargo loading	Pre-loading (genetic over-expression in parent cells); post-loading (electroporation, sonication, chemical conjugation); EV-liposome hybrids	Enrich therapeutic RNAs/drugs; programmable gene modulation	Gene knock-down/ translation in target cells; improved mRNA delivery with hybrids	Electroporation-induced aggregation; low loading efficiency; cargo leakage; vesicle damage; endotoxin carry-over; scalability limits	[73–75]
Parental cell programming	CRISPR/viral engineering; culture optimisation (hypoxia, inflammatory licensing)	Tailor EV cargo; innate tropism/targeting	Cardiomyocyte-homing EVs; improved cardiac uptake/ function	Off-target genomic effects; vector-integration risks; phenotype drift; comparability bridging required	[18,72]
Biomaterial integration strategies					
Matrix immobilisation	Natural vs synthetic matrices; CD63-binding peptide (CP05) tethers	Spatiotemporal control; stability enhancement	Controlled release kinetics; preserved EV bioactivity post-delivery	Unintended sequestration/hindered release; cross-linker toxicity; sterilisation and resorption control	[77]
3D bioprinting integration	Bioinks (hydrogel + EVs) for spatial patterning/gradients	Tissue-conformal placement; gradient delivery	Cartilage/myocardial constructs: spatial EV retention; improved function	Shear-related EV damage/nozzle clogging; deposition reproducibility; potential combination-product classification	[77]
Manufacturing and scale-up approaches					
TFF/continuous processing	Closed-loop concentration/ diafiltration; continuous, closed processing	Scalable, gentle purification; high recovery	High yield (particles/μg protein); maintained potency vs reference lot; reduced processing time	Membrane fouling; shear stress; product loss during diafiltration; integrating viral-clearance steps	[16]
Hollow-fibre bioreactors	Perfused, high-surface-area reactors for continuous MSC culture/EV harvest	High-density upstream; scalable EV yield	High productivity (particles L^{-1} day^{-1}); consistent EV size/markers	Channel fouling; oxygen/nutrient gradients; contamination risk; cleaning validation; scale-up complexity	[17]
Microfluidics/in-line QC	SEC chips; immunocapture; on-chip analytics; Raman-based monitoring	High purity; automated, closed-loop QC	Purity indices; reduced hands-on time; GMP readiness	Throughput limits; channel clogging; calibration variability; GMP validation burden	[76]
Regulatory considerations					
Regulatory/CMC guidance	ISEV/ISCT frameworks; MISEV2018/2023	Standardisation; comparability; GMP compliance	Adherence to reporting guidelines; comprehensive CMC documentation for trials	Inter-regional classification variance; evolving guidances; potency-assay validation; lot comparability evidence; raw-material traceability	[19,25,26]

BD, biodistribution; CMC, chemistry-manufacturing-controls; dECM, decellularised extracellular matrix; EV, extracellular vesicle; GelMA, gelatin methacryloyl; GMP, Good Manufacturing Practice; IN, intranasal; IV, intravenous; MI, myocardial infarction; MPS, mononuclear phagocyte system; MSC-EV(s), mesenchymal stromal cell-derived extracellular vesicle(s); NHP, non-human primate; PCL, polycaprolactone; PEG, polyethylene glycol; PK, pharmacokinetics; PVA, poly(vinyl alcohol); QC, quality control; RGD, arginine-glycine-aspartic acid peptide; RVG, rabies virus glycoprotein peptide; SEC, size-exclusion chromatography; SPION, superparamagnetic iron-oxide nanoparticle; TFF, tangential-flow filtration.

Table 3
Clinical and preclinical applications of MSC-EVs.

Indication	Study type	MSC-EV source	Delivery/platform	Dose metric and amount	Assessment window	Key outcomes (representative)	Key references
Chronic wounds	Preclinical (rodent)	UC-MSC, AD-MSC	Topical hydrogels (GelMA; O_2-nanobubble hydrogel) or local injection	–	–	Faster closure; ↑ capillary density; improved collagen organisation; oxygenated nanobubble carriers enhanced EV uptake & wound healing	[84]
Diabetic foot ulcers (chronic, non-healing)	Clinical (registered; recruiting)	WJ-MSC (sEV)	Topical gel application	sEV topical; dose NR	Primary wound-healing readouts typically 4–12 weeks	Ongoing evaluation; no peer-reviewed outcomes yet. Pooled human EV studies show low serious AEs overall.	[ClinicalTrials.gov NCT06812637; 85, 88]
Knee osteoarthritis	Clinical (triple-blind RCT)	Placental MSC	Intra-articular (single dose)	5 mL per knee (particles NR)	6 months (WOMAC, VAS, MRI)	Safe; no superiority vs placebo at 6 months across prespecified endpoints	
Bone defect regeneration	Preclinical (rodent)	BM-MSC, UC-MSC	EV-functionalised scaffolds	–	–	↑ bone volume; ↑ vessel density; ↑ biomechanical strength	[89]
Tendon/ligament repair	Preclinical (rodent)	BM-MSC, AD-MSC	Local (EV-conditioned microenvironment)	–	–	Early-passage EVs > senescent EVs for collagen alignment & tensile strength	[90]
Stroke/TBI	Preclinical (rodent)	BM-MSC, UC-MSC	Intranasal (vs IV)	–	–	↓ neuroinflammation; improved BBB integrity; functional recovery; route-dependent effects (IN vs IV)	[65,91]
Myocardial infarction	Preclinical (rodent)	BM-MSC, AD-MSC	IV or intramyocardial; targeted depots	–	–	↓ infarct size; preserved LVEF; greater effect with engineered targeting	[66,69,70]
ARDS (COVID-19)	Clinical (Phase II RCT, N = 102)	BM-MSC	IV (two doses)	High-dose cohort: 15 mL on Days 1 & 2	60-day mortality; ventilator-free days	Acceptable safety; overall neutral primary endpoint; exploratory signal in 18–65 y at 15 mL × 2 (↓ mortality; ↑ VFDs)	[86]
ARDS (COVID-19)	Clinical (double-blind RCT, N ~ 45)	Placental MSC	IV adjunct to SOC	Dose NR (IV sEVs)	In-hospital mortality; respiratory indices	Lower in-hospital mortality vs placebo; improved respiratory indices	[96]
COVID-19 pneumonia	Clinical (pilot)	AD-MSC; UC-MSC	Nebulised inhalation	Up to ~2 × 10^9 vesicles over 5 days (pilot, n = 7)	7–30 days (clinical/CT)	Feasibility/tolerability; improved oxygenation/CT; no EV-related AEs reported	[97,98]
Chronic cough after COVID-19	Clinical (registered; ongoing)	UC-MSC	Nebulised inhalation	Nebulised EVs; dose NR	NR	Ongoing clinical evaluation	[ClinicalTrials.gov NCT05808400]
Acute kidney injury	Preclinical (mouse)	BM-MSC	IV	–	–	Improved renal function and survival	[99]
Chronic kidney disease	Clinical (pilot)	UC-MSC	IV (±intrarenal)	100 μg EV protein/kg weekly × 2	Short-term renal indices	Feasibility; transient ↑ GFR and ↓ proteinuria	[100]
Liver fibrosis	Preclinical (rodent meta-analysis)	Various MSC sources	IV (typical)	–	–	↓ collagen deposition; improved histological fibrosis scores	[101]
Inflammatory bowel disease	Preclinical (mouse)	BM-MSC	Intraperitoneal	–	–	Reduced colitis via EV-borne TSG-6; enhanced barrier repair	[103]
Amyotrophic lateral sclerosis (ALS)	Clinical (pilot, open-label)	BM-MSC	IV (two infusions)	10 mL × 2, one month apart	3 months (ALSFRS-R)	No treatment-related SAEs; 30 % with non-decline in ALSFRS-R over 3 months; supports RCT progression	[95]

AD-MSC, adipose-derived MSC; AE, adverse event; ALSFRS-R, ALS Functional Rating Scale–Revised; ARDS, acute respiratory distress syndrome; BBB, blood–brain barrier; BM-MSC, bone-marrow MSC; EV, extracellular vesicle; GelMA, gelatin methacryloyl; GFR, glomerular filtration rate; IA, intra-articular; IN, intranasal; IV, intravenous; LVEF, left ventricular ejection fraction; MSC-EV(s), mesenchymal stromal cell-derived extracellular vesicle(s); NR, not reported; RCT, randomised controlled trial; SOC, standard of care; sEV, small extracellular vesicle; TBI, traumatic brain injury; TSG-6, tumour necrosis factor-stimulated gene-6; UC-MSC, umbilical-cord MSC; VAS, visual analogue scale; VFDs, ventilator-free days; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

analytical platform, and the markers used for identification—both positive (e.g., CD9, CD63, CD81, TSG101, ALIX) and at least one negative marker (e.g., calnexin, GM130) [25,26]. Fig. 1 illustrates the principal pathways of EV biogenesis and release.

3.2. Composition and cargo of MSC-derived EVs

MSC-EVs transport proteins, lipids, nucleic acids and metabolites that reflect the source tissue, donor attributes and conditioning of their parent MSCs [29,30].

- Membrane components: Tetraspanins (CD9, CD63, CD81), integrins (e.g., CD29/β1, CD51/β5) and adhesion molecules (ICAM-1, VCAM-1) are abundant and can influence tissue tropism and cellular uptake [31,32].
- Proteins: Heat-shock proteins (HSP70/90), metabolic enzymes, cytokines (e.g., transforming growth factor-β (TGF-β), IL-6), growth factors (vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF)) and ECM-modifying enzymes (e.g., matrix metalloproteinases) are common. Endosomal sorting proteins (ALIX, TSG101) and Rab GTPases often co-isolate, serving as markers of endosomal origin [31,33].

Table 4
Translational gaps and enabling solutions for MSC-EV therapeutics (decision matrix).

Focus area	Specific gap/challenge	Significance	Near-term actions (1–2 years)	Mid-term enablers	Owner (primary stakeholders)	References
Definitions and reporting	Variable nomenclature; uneven adherence to MISEV reporting	Confounds comparability; impedes meta-analysis and peer review	Adopt MISEV2018/2023 checklists in SOPs and methods; declare operational EV definition and isolation method in every section that uses subtype terms	Journal/registry enforcement; community templates (EV-TRACK)	ISEV/ISCT; journal editors; trial registries	[25,26, 111]
Mechanisms	Incomplete maps of cargo sorting/ uptake	Hinders potency-assay design and dose selection	Mechanism-linked in-vitro panels per indication (e.g., T-cell suppression; M1→M2; HUVEC tube formation)	Perturbation-driven multi-omics causal maps	Academic consortia; core facilities; funders	[110,111]
Dose metrics & reporting	Heterogeneous units (particles, μg protein, "cell-equivalents"); weak exposure-response	Obscures pharmacology; risks mis-dosing and non-reproducible trials	Dual-metric dosing (particles + protein; optional cell-equivalents) paired with a potency surrogate; pre-specify assessment windows; route-aware exposure metrics (e.g., MPS uptake for IV; depot release t½ for hydrogels; brain %ID/g for IN; inhaled/retained fraction for nebulised)	Route-aware PK/BD/PD modelling and dose-response across regimens; field consensus on minimal reporting set	Developers; biostatisticians; ISEV/ ISCT dose-reporting WG	[111,114]
Potency	No validated, indication-relevant potency assays for lot-release	Lot-release & comparability at risk; weak predictive value	Minimal lot-release panel: identity (CD9/CD63/CD81/TSG101/ ALIX) + one negative marker (e.g., calnexin/GM130); size/ concentration (calibrated); sterility/endotoxin; mycoplasma; particle:protein trending. Indication-aligned development panel with acceptance criteria: Anti-inflammatory—CFSE T-cell suppression; M1→M2 (IL-6/IL-10, ARG1/CD206). Pro-angiogenic—HUVEC tube formation; scratch-closure. Anti-fibrotic—TGF-β myofibroblast suppression (α-SMA, *p*-SMAD2/3; COL1A1/3).	Inter-lab ring trials; shared reference EVs and pre-defined acceptance ranges; composite potency indices benchmarked to in-vivo outcomes	Developers; ISCT-ISEV taskforce; regulators	[116]
CMC & comparability	Platform changes (2D→3D/HFB; media swaps; UC→TFF/SEC) alter EV attributes	Alters efficacy/safety; dossier uncertainty	Lock a primary isolation platform (TFF ± SEC); define CQAs and acceptance ranges; pre-specify a comparability plan and bridging criteria (identity + potency + PK/BD labels) for any change (bioreactor, media, TFF membrane, licensing/hypoxia set-points)	Inline/at-line analytics; QbD control strategies; continuous processing; multi-attribute methods	Developers; CDMOs; Quality units	[16,112]
Stability	Cryo/lyo protocols not harmonised; freeze-thaw sensitivity	Potency loss; cost/logistics burden	Trehalose/sucrose-buffered lyophilisation with accelerated/real-time stability; release limits for size, potency and purity post-reconstitution	Pharmacopeial methods; stability-indicating functional assays	Developers; pharmacopeias (USP/Ph. Eur.); CDMOs	[118]
Safety	Long-term immunogenicity/ thrombogenicity unclear; risk with engineered surfaces/repeat dosing	Critical for chronic indications and repeat exposure	GLP Safety & Immunogenicity panel: Biodistribution/PK (labelled EVs, route-matched); Thrombogenicity/coagulation (TF activity; thrombin-generation; TAT complex; platelet activation); Complement activation (CH50/AH50; C3a, C5a, sC5b-9); Cytokine-release in human whole blood; haemocompatibility; anti-PEG/anti-EV antibodies; repeat-dose immunogenicity/ABC risk; age/ comorbidity strata	Registries & pharmacovigilance; scenario-specific escalation for engineered/targeted EVs; device-related risk (e.g., nebulisers)	Developers; CROs; regulators	[85,112, 113]
Targeting	Limited tropism/retention at lesion sites	Drives higher doses; off-target uptake	Surface-ligand strategies (e.g., RGD, RVG); route-appropriate depots/scaffolds	Dual-targeting and stimuli-responsive designs	Developers; academic labs	[119,120]
Gene editing	Inconsistent CRISPR loading/ activity; off-target risk	High regulatory bar; complex CMC	Standardise loading QC; orthogonal off-target screening; EV/LNP hybrids as bridging	Scalable CRISPR-EV production with validated biodistribution	Developers; gene-therapy consortia; regulators	[121–125]
Regulation	Divergent regional classification and expectations	Adds cost; dossier complexity	Early scientific advice; pathway mapping; align potency & comparability with MISEV and biologics guidance	International harmonisation; cross-agency workshops	Regulators (EMA/FDA/ PMDA); developers; societies	[19]
Ecosystem	Fragmented methods; limited training and shared assets	Redundant validation; slow convergence	Shared SOPs; reference EVs; open datasets; community proficiency testing	Multi-site consortia and training hubs	ISEV/ISCT; journals; funders	[111]

ABC, accelerated blood clearance; BD, biodistribution; CDMO, contract development and manufacturing organisation; CQA, critical quality attribute; EV, extracellular vesicle; GLP, Good Laboratory Practice; HFB, hollow-fibre bioreactor; ISEV/ISCT, International Societies for Extracellular Vesicles/Cell & Gene Therapy; LNP, lipid nanoparticle; lyo, lyophilisation; MISEV, Minimal Information for Studies of Extracellular Vesicles; MOA, mechanism of action; PD, pharmacodynamics; PK, pharmacokinetics; QbD, Quality by Design; SEC, size-exclusion chromatography; SOP, standard operating procedure; TAT, thrombin-antithrombin; TFF, tangential-flow filtration; TF, tissue factor.

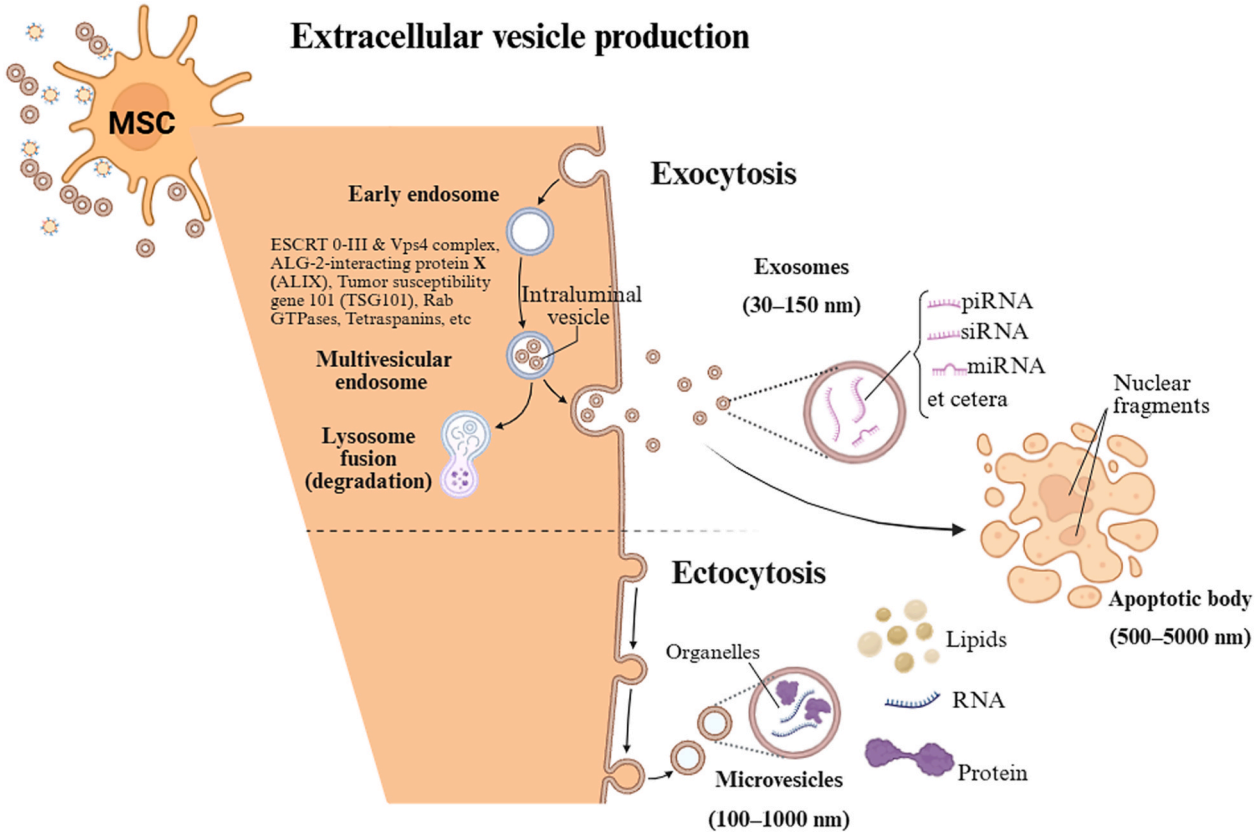

Fig. 1. Biogenesis and release pathways of MSC-EVs. Schematic of the principal EV subtypes released by MSCs—small EVs of endosomal origin (often termed exosomes), microvesicles (ectosomes; budding from the plasma membrane) and apoptotic bodies (released during apoptosis), generated via exocytosis or ectocytosis. Key molecular regulators—such as ESCRT complexes, Rab GTPases, ceramides and tetraspanins—are indicated alongside typical size ranges and cargo profiles for each vesicle class. *The figure created with* BioRender.com*, accessed on November 20, 2025*.

- Nucleic acids: EVs contain mRNAs and multiple classes of non-coding RNAs, notably microRNA (miRNAs) (e.g., miR-21, miR-126, miR-146a), long non-coding RNAs and circular RNA (circRNAs), which regulate inflammation, angiogenesis and differentiation [34]. EV-associated DNA has also been reported, but its abundance and function remain under active investigation, and may reflect intravesicular or surface-associated nucleic acid [33].
- Lipids: EV membranes are enriched in sphingomyelin, ceramides, cholesterol and phosphatidylserine, conferring stability and supporting receptor-independent signalling and uptake [29,30].
- Metabolites: MSC-EVs carry a variety of metabolites (e.g., amino acids, organic acids and lipids) that can modulate the metabolic state of target cells, reflecting the parent cell's metabolism and culture conditions [30].

Collectively, this multimodal cargo underpins canonical regenerative functions (immunomodulation, angiogenesis, antifibrosis and cytoprotection) elaborated in Section 4.

3.3. Source-dependent variability

MSC tissue of origin and donor attributes strongly influence EV composition and bioactivity. For example, adipose MSC-EVs often carry pro-angiogenic factors such as VEGF, HGF and miR-126 that promote neovascularisation [31]. Bone-marrow MSC-EVs may be enriched in osteogenic and haematopoietic regulators, containing higher levels of pro-differentiation and chemotactic proteins that support osteogenesis and haematopoiesis [32]. Umbilical-cord MSC-EVs commonly display pronounced immunomodulatory profiles [32,34].

Donor cell age and condition also impact EV cargo. Senescent MSCs release EVs skewed towards pro-inflammatory and pro-fibrotic signatures, mirroring aspects of the senescence-associated secretory phenotype (SASP)—a state in which senescent cells secrete inflammatory cytokines, chemokines and proteases [35]. Furthermore, technical factors such as the EV isolation method and environmental conditions (e.g. hypoxic culture) can introduce additional variability in EV yield and content [36,37]. Accordingly, programmes should prospectively record donor characteristics (age/health/sex), passage range and conditioning parameters to enable comparability across experiments [25,26].

3.4. Methodological considerations

Technical and biological parameters influence MSC-EV yield, purity and function [25,26]. Key variables include.

- Isolation and purification: Ultracentrifugation (UC)can co-pellet lipoproteins and protein contaminants, whilst size-exclusion chromatography (SEC) improves purity. TFF is scalable, and SEC-plus-TFF "polishing" workflows achieve high EV yields without sacrificing purity [36]. Where clinical use is envisaged, processes should be locked into GMP-compatible, closed systems with pre-defined comparability criteria [38].
- Culture media: Foetal bovine serum (FBS) introduces bovine EVs and RNAs; thus, serum-free or xeno-free media are preferred (though they may alter MSC growth and EV cargo). Human platelet lysate offers a promising alternative but requires rigorous EV depletion to avoid platelet-EV contamination [25,26].
- Cell state: Passage number, metabolic activity and exposure to stimuli (e.g., hypoxia, inflammatory cytokines, mechanical stress) modulate EV yield and potency [31,34]. For example, hypoxic

preconditioning can increase EV production and selectively enrich angiogenic miRNAs (such as miR-210) via hypoxia-inducible factor-1α (HIF-1α)-dependent packaging pathways [37]. The miRNA cargo of MSC-EVs is itself a key mediator of their regenerative effects; EV-borne miRNAs have been shown to enhance wound healing by reprogramming target cells in injury models [39]. Likewise, inflammatory priming with interferon-γ (IFN-γ) or tumour necrosis factor-α (TNF-α) induces the release of EVs enriched in immunoregulatory molecules (indoleamine 2,3-dioxygenase (IDO), TSG-6, programmed death-ligand (PD-L1)), bolstering their anti-inflammatory potential [40,41]. Standardising these parameters (e.g., using early-passage, pre-specified conditioning) is critical to ensure consistent EV production and functionality [25,26].

- Quantification and dose reporting: Report EV quantity using at least two orthogonal metrics (e.g., particles and protein), and specify the instrument/model, analytical settings and calibration standards used for measurement [25,26].
- Analytical controls: Use nuclease/protease treatments with and without mild detergents, spike-in controls and appropriate buffer blanks to confirm intravesicular cargo and to exclude co-isolated contaminants or aggregation artefacts [25,26]. Apply at least one negative-marker immunoblot (e.g., calnexin/GM130) on process-matched fractions to verify minimal cellular contamination, in line with MISEV2023 guidance [14,25,26].

4. Regenerative mechanisms of MSC-EVs

MSC-EVs recapitulate many of the regenerative functions of their parental cells through diverse mechanisms, notably immunomodulation, pro-angiogenic signalling, anti-fibrotic activity, cytoprotection and targeted delivery of bioactive cargo [38]. These nanoscale vesicles convey a broad spectrum of molecular signals that modulate immune responses, promote vascular and tissue repair, and attenuate pathological fibrosis in injured or diseased tissues. The therapeutic efficacy of MSC-EVs depends on efficient cargo delivery and the functional integration of transferred biomolecules within recipient cells. In practice, these mechanisms operate concurrently and are dose-, route- and context-dependent, so multiple concurrent EV-mediated signals are typically involved. Accordingly, mechanistic readouts (e.g., M1→M2 polarisation, endothelial tube formation, α-SMA/collagen suppression) can serve as mechanism-linked potency surrogates (see Table 1).

MSC-EVs exert their therapeutic effects through two primary modes: (i) direct regenerative signalling to tissue-specific cells, and (ii) indirect modulation of the injury microenvironment through immune and vascular reprogramming. This dual approach enables MSC-EVs to address intertwined pathological processes and coordinate the cellular cross-talk necessary for effective tissue repair.

4.1. Immunomodulation

MSC-EVs exert profound immunomodulatory effects on innate and adaptive immune cells, creating a reparative microenvironment through multiple complementary mechanisms. They suppress excessive inflammation by reducing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-17) and chemokines. At the same time, they increase levels of anti-inflammatory mediators such as IL-10 and interleukin (IL)-1 receptor antagonist (IL-1RA) [39].

4.1.1. Macrophage polarisation and innate immunity

MSC-EVs promote macrophage polarisation from a pro-inflammatory (M1) to an anti-inflammatory (M2-like) phenotype. One mechanism involves EV-associated CD73, which generates adenosine to activate A_2A/A_2B adenosine receptors on macrophages. Blocking CD73 or adenosine signalling attenuates this effect. EV cargo such as miR-146a suppresses NF-κB signalling by targeting IRAK1 and TRAF6. Additionally, EV-associated TGF-β can modulate NK-cell cytotoxicity through SMAD2/3 signalling [40].

4.1.2. Adaptive immune regulation

MSC-EVs regulate T- and B-cell function via surface proteins and miRNAs. Cytokine-primed MSCs secrete EVs enriched in PD-L1/PD-L2, which engage PD-1 on T cells, suppressing effector proliferation and promoting regulatory T cell (Treg) expansion [41]. In murine graft-versus-host disease (GvHD), blockade of PD-L1 negated EV therapeutic effects [42]. EV-derived miRNAs also inhibit dendritic-cell maturation and T-cell activation [40]. EV-associated ecto-enzymes (CD39/CD73) generate adenosine, further dampening Th1 effector activity [43].

4.1.3. Systemic anti-inflammatory effects

MSC-EVs often carry miRNAs (miR-146a-5p, miR-21, miR-223, let-7 family) that collectively down-regulate NF-κB and inflammasome activation. In acute respiratory distress syndrome (ARDS), EV-derived miR-181a-5p reprogrammed macrophages via the PTEN/STAT5/SOCS1 axis, promoting resolution [42]. In autoimmune hepatitis, MSC-EVs lowered the Th17/Treg ratio by delivering miR-223 and miR-1246 [39]. Immunomodulatory magnitude varies by MSC source, licensing state and dose; mechanism-linked assays are needed for reproducibility [39–41].

4.2. Angiogenesis and vascular regeneration

MSC-EVs stimulate endothelial proliferation, migration and tube formation, enhancing perfusion in ischaemic tissues [43].

4.2.1. Pro-angiogenic cargo and signalling

MSC-EVs deliver VEGF, FGF-2, platelet-derived growth factor and angiopoietins to vascular cells, upregulating VEGFR2 and downstream AKT/ERK signalling [43]. EV miRNAs including miR-21, miR-23a, miR-125a and miR-31 suppress anti-angiogenic genes and promote endothelial sprouting [40,44].

4.2.2. Mechanisms of vascular network formation

MSC-EVs support angiogenesis, vasculogenesis and arteriogenesis. For instance, bone marrow MSC-EV miR-486 targeted PTEN, thereby activating the AKT pathway and increasing angiogenesis post-stroke [45]. EVs also mobilise monocytes, which release angiogenic factors to support collateral artery formation [43]. Reported pro-angiogenic effects are context-dependent and influenced by EV source, dose and route [43,44].

4.3. Anti-fibrotic mechanisms

MSC-EVs mitigate fibrosis across multiple organs by inhibiting fibroblast activation and excessive extracellular matrix (ECM) deposition [46].

Targeting TGF-β/SMAD: EV miRNAs (miR-21-5p, miR-125b, miR-23a-3p) block SMAD2/3 phosphorylation, reducing myofibroblast transdifferentiation [40].

miR-29 family: directly silences collagen transcripts, limiting ECM accumulation [46].

Lung fibrosis: EV miR-4516 suppressed integrin αV, reducing TGF-β activation [47]; umbilical cord MSC-EV miR-486-5p promoted M2 macrophage polarisation, alleviating fibrosis [48].

4.4. Cytoprotection and cell survival

MSC-EVs transfer anti-apoptotic proteins (Bcl-2, survivin, heat-shock proteins (HSPs)) and miRNAs (miR-21, miR-19a, miR-199a-3p) that activate phosphoinositide 3-kinase (PI3K)/AKT and ERK pathways, reducing apoptosis [40]. In cardiac injury, EV-delivered miR-21 suppressed PTEN, decreasing infarct size [40].

4.4.1. Mitochondrial transfer

MSC-EVs may deliver mitochondrial proteins, mitochondrial DNA (mtDNA), or even entire mitochondria, supporting bioenergetic rescue in stressed cells [49]. EVs have been observed to transfer mitochondrial content to neutrophils in inflamed liver, restoring their function and reducing neutrophil extracellular trap (NET) formation [39,50].

4.5. Cargo delivery and functional integration

MSC-EVs are natural delivery vehicles; their membranes protect RNA cargo, ensuring stability in circulation [28].

Uptake pathways: clathrin- and caveolin-mediated endocytosis, macropinocytosis and phagocytosis [51].

Targeting: EV surface integrins (e.g., α6β4/α6β1, αvβ5) direct vesicle uptake into specific organs (α6β4/α6β1 to the lung, αvβ5 to the liver) [28].

Cargo diversity: EVs carry non-coding RNAs, proteins, mRNAs and lipids, which collectively orchestrate immunomodulatory, angiogenic and anti-fibrotic responses in recipient cells [40].

4.6. Integrated regenerative signalling

MSC-EVs support tissue regeneration through coordinated direct and indirect mechanisms that often act synergistically. Direct signalling entails delivery of bioactive EV cargo into parenchymal cells (e.g., cardiomyocytes, neurones, epithelial cells). Indirect signalling reprogrammes immune and stromal compartments to establish a pro-regenerative niche [45,50]. The relative importance of these pathways varies by tissue type, injury severity, and timing of MSC-EV administration. This variability reinforces the need for prospectively defined, mechanism-linked potency assays to guide development (see Table 1).

5. Source considerations and culture conditions

The origin and conditioning of MSCs profoundly influence the molecular composition, biological activity, and translational suitability of their EVs. Systematic differences arise from MSC tissue of origin, donor characteristics, and culture parameters, each of which can affect EV yield, cargo composition, and therapeutic potency [18]. These determinants should guide therapeutic targeting, bioprocess design, and safety profiling strategies in MSC-EV development. Understanding source-dependent variability is essential for the rational design of EV-based therapeutics, as different MSC origins confer distinct regenerative and immunomodulatory profiles that translate into divergent therapeutic effects in vivo [28]. However, direct comparisons remain challenging owing to methodological heterogeneity in EV isolation techniques, characterisation methods, and potency assays. Accordingly, programmes should prospectively capture key source variables (donor age/sex/health status), passage range/population-doubling level, and conditioning parameters to enable comparability across studies.

5.1. Comparison of MSC sources and their EV characteristics

5.1.1. Bone-marrow-derived MSCs (BM-MSCs)

Bone marrow MSC-EVs are the most extensively characterised in clinical contexts, especially for immune-mediated disorders and tissue repair. For example, in steroid-refractory GvHD, compassionate-use bone marrow MSC-derived exosomes led to marked improvements in cutaneous and mucosal manifestations [10]. Mechanistically, these vesicles carry immunosuppressive factors (e.g., TGF-β, IL-1RA) and regulatory miRNAs, and can engage adenosinergic signalling via CD39/CD73 ecto-enzymes [31,34]. However, donor age and cellular senescence can alter the MSC phenotype and EV cargo; MSC-EV products from older donors tend to exhibit pro-inflammatory shifts and reduced regenerative efficacy [52,53]. Cross-study variability likely reflects both source biology and preconditioning/bioprocess differences; standardised, mechanism-of-action-linked release assays are needed for comparability [15].

5.1.2. Adipose-tissue-derived MSCs (AD-MSCs)

Adipose-derived MSCs are abundant and highly secretory, producing EVs enriched in pro-angiogenic and anti-inflammatory cargo. In soft-tissue repair and wound-healing models, adipose-derived MSC-EVs promote M1→M2 macrophage polarisation, accelerate re-epithelialisation, and enhance neovascularisation [53]. These EVs are frequently enriched in angiogenic mediators such as VEGF and miR-126, supporting their use in cardiovascular and ischaemic applications [53]. In chronic wounds, adipose-derived MSC-EVs have been reported, mainly in preclinical models, to influence multiple phases of healing, including inflammatory resolution and remodelling [53]. Head-to-head source comparisons remain limited; conclusions should be interpreted alongside EV dose unit(s), route, and timing [53].

5.1.3. Umbilical-cord-derived MSCs (UC-MSCs)

Perinatal MSCs (e.g., Wharton's jelly) show high proliferation, low immunogenicity, and potent secretomes. Umbilical cord MSC-EVs are strongly pro-angiogenic, in part via Wnt/β-catenin signalling; for example, EV-mediated transfer of Wnt4 into endothelial cells promotes proliferation and tubulogenesis, whereas Wnt4 knock-down in parental MSCs attenuates these effects [54]. Pragmatically, umbilical cord MSC sources scale well for EV manufacturing: microcarrier-based three-dimensional (3D) culture combined with TFF can yield substantially higher quantities of functionally active EVs than conventional two-dimensional (2D) methods [55], and can be implemented within closed, GMP-compatible workflows.

5.1.4. Specialised and emerging sources

Dental-pulp MSCs (DP-MSCs) secrete neurotrophic EVs; preclinical studies indicate neuroprotective effects in neural injury models [56]. In ocular models, bone marrow MSC-derived exosomes improved retinal ganglion cell survival and function [57].

Placental/amnion MSCs provide a scalable, low-immunogenic EV source with combined anti-fibrotic and anti-inflammatory effects [58]. Menstrual-blood MSCs (MenSCs) are readily accessible; MenSC EVs display strong immunomodulatory activity, which can be enhanced by cytokine licensing [59].

Hair-follicle-derived MSCs yield EVs that support dermal repair and angiogenesis in preclinical settings; comparative potency versus adipose-derived MSC-EVs remains to be defined [53,58].

Induced-pluripotent-stem-cell-derived MSCs (iMSCs) offer an effectively unlimited cell supply, but lot-to-lot functional variability has been reported; rigorous safety screening (e.g., for residual pluripotent cells) is essential when preparing iMSC EVs [58].

Emerging sources are promising but require assay-linked potency data and aligned dose units before reliable cross-source ranking [15,53].

5.2. Donor-dependent factors and preconditioning

5.2.1. Age and health status

Donor age and overall health status influence EV cargo and function. Ontogeny/ageing can shift MSC-EV profiles towards pro-inflammatory mediators and diminish regenerative potency [52,53]; age-linked miRNA changes in MSC-EV products have also been described [60]. Early-passage MSCs tend to produce EVs enriched in reparative factors, whereas late-passage/senescent MSCs secrete EVs with more catabolic signatures [60,61]. Age effects interact with culture conditions; reporting passage, population-doubling level, and senescence markers is recommended [61]. Where feasible, also capture donor sex, body mass index (BMI)/metabolic syndrome, smoking status, and relevant medications, as these can plausibly modulate EV potency.

5.2.2. Inflammatory licensing

Preconditioning MSCs with pro-inflammatory cytokines (e.g., IFN-γ, TNF-α, IL-1β) ("licensing") can increase the immunosuppressive potency of secreted EVs. Licensed MSC-EVs often express higher levels of IDO and PD-L1, enhancing T-cell suppression and Treg induction [41]; where tested in vitro, blockade of these axes attenuates EV-mediated T-cell suppression. Standardising licensing protocols (cytokine, dose, duration) and documenting carry-over/clearance and washout is necessary for reproducibility [61].

5.2.3. Hypoxic preconditioning

Exposing MSCs to hypoxia (typically 1–3 % O_2 for 24–48 h) boosts EV secretion and enriches HIF-1α-regulated cargo (e.g., miR-210, miR-205-5p), augmenting angiogenic and cytoprotective efficacy [56]. In myocardial infarction models, hypoxia-preconditioned adipose-derived MSC-EVs reduced reactive oxygen species (ROS) and inhibited ferroptosis via a circular RNA (circRNA)/miR-15a/glutathione peroxidase 4 (GPX4) axis; genetic HIF-1α stabilisation in MSCs similarly increased protective EV bioactivity [56]. Programmes should specify oxygen-tension set points, measurement method, and exposure duration to support comparability.

5.3. Novel mechanisms: mitochondrial transfer and metabolic modulation

Beyond canonical RNA and protein signalling, MSCs can export mitochondria or mitochondrial components within EVs. Tissue-derived mitochondria-rich EVs ("mitoEVs") have been reported to transfer respiratory-competent mitochondria and to enhance bioenergetic and reparative functions in recipient cells in preclinical models [49,50,57]. Mitochondrial transfer via EVs is compelling but requires orthogonal controls to exclude co-isolated organelles and to clarify dose-response [50], for example nuclease/protease treatments with/without mild detergents, density-gradient separation, and high-resolution (cryo-)electron microscopy.

5.4. Culture optimisation and emerging technologies

5.4.1. Three-dimensional (3D) culture systems and bioprocessing

Three-dimensional (3D) systems (e.g., hollow-fibre bioreactors, stirred-tank reactors, spheroids) generally increase EV productivity per cell compared with 2D monolayers, and dynamic culture conditions (shear, cell–cell contacts) can modulate EV yield and cargo composition. Hollow-fibre/3D platforms integrated with TFF support scalable, clinically oriented EV manufacture [58]. Productivity gains must be weighed against shifts in cargo and function; in-process bridging comparability (pre-defined CQAs and potency) is advised [38].

5.4.2. Media optimisation and xeno-free production

Serum-free/xeno-free or chemically defined media reduce bovine EV contaminants and simplify downstream purification. Several serum-free/xeno-free or chemically defined formulations maintain or even increase EV yields compared with FBS-containing media, whilst improving regulatory compliance [38,59]. Media swaps can alter EV cargo; bridging studies should link EV analytical attributes to mechanism-linked potency [11,15]. Deplete any human platelet lysate or supplements of endogenous EVs to minimise co-isolation, and report EV yield as particles per 10^6 cells per 24 h together with particle:protein ratios for batch trending.

5.4.3. Artificial intelligence/machine learning-driven process optimisation

Emerging artificial intelligence/machine-learning (AI/ML) approaches are being explored conceptually to optimise MSC-EV production and quality monitoring, for example through data-driven adaptive control strategies informed by multi-parametric sensor data. These strategies remain at an early, exploratory stage and would require transparent models, robust cross-platform validation and predefined safety bounds before routine implementation.

5.5. Source-indication matching: towards precision EV therapeutics

Pragmatic source selection should integrate intrinsic potency, manufacturability, immunogenicity, regulatory acceptability, and process standardisation. Emerging data suggest that inflammatory-licensed, especially IFN-γ-primed, bone-marrow MSC-EVs show enhanced immunosuppressive activity in autoimmune and inflammatory models [40,41,60,61], whereas adipose- and umbilical-cord-derived MSC-EVs—particularly when produced under hypoxic conditions—have been preferentially evaluated in ischaemic and chronic wound-healing settings [18,37,43,53]. However, robust predictive criteria are not yet established; harmonised dose units, route-dependent biodistribution, and exposure-response data are required to move from plausibility to precision. A comprehensive decision matrix linking MSC sources, target indications, and associated bioprocess strategies is provided in Supplementary Table S1. For reproducibility and regulatory readiness, align source choice with a prospectively defined potency panel and a prespecified comparability plan (see Supplementary Table S1).

6. Innovative engineering and delivery platforms

6.1. Route-first delivery strategies for MSC-EV therapeutics

6.1.1. Intravenous (IV)

IV administration is the most studied systemic route but is limited by rapid mononuclear phagocyte system (MPS) clearance—predominantly hepatic and splenic—constraining exposure to minutes [62]. Repeat dosing may alter clearance kinetics; in particular, PEGylation can induce anti-polyethylene glycol (PEG) antibodies and accelerated blood clearance on re-exposure [63]. For robust cross-study comparability and exposure–response analyses, IV studies should quantify early MPS uptake (e.g., hepatic/splenic fractions), report dual dose units (particles plus protein), pre-specify distribution versus clearance sampling windows, and include complement/coagulation safety evaluations when appropriate [62,63].

6.1.2. Local injection (intramuscular, intra-articular, intramyocardial)

Local delivery increases target-site exposure, although EVs may still diffuse or drain via lymphatics [62]. A 2024 randomised trial of a single high-dose intra-articular (IA) injection for knee osteoarthritis confirmed safety but no superiority over placebo at 6 months [64], motivating evaluation of repeat dosing and/or sustained-release depots. Local administration studies should document targeting method, local retention/dispersion, the rationale for single versus repeat dosing schedules, and pre-specified pain/function endpoints with defined assessment windows [62,64].

6.1.3. Intranasal (nose-to-brain)

Intranasal (IN) delivery can bypass the blood–brain barrier (BBB) via olfactory and trigeminal pathways. In rodent stroke and traumatic brain injury (TBI) models, IN MSC-EVs reached brain parenchyma and improved neurofunctional outcomes; however, superiority versus IV is not consistent across endpoints, indicating route- and model-dependent effects [62,65]. IN studies should report regional brain uptake (e.g., percentage injected dose per gram or normalised signal), neurobehavioural or biomarker assessment windows, and controls that distinguish nasal deposition from pulmonary spill-over [65].

6.1.4. Inhalation/nebulised delivery

Non-invasive pulmonary delivery is under active study for cardiac and respiratory indications. Nebulised MSC-EVs administered after myocardial infarction homed to injured myocardium and improved function in mouse and pig models [66]. Aerosol studies should

characterise EV integrity pre- and post-device, regional lung deposition, inhaled/retained dose normalisation, and systemic exposure [66]. Where clinical translation is intended, device–EV interaction (shear/-aerosolisation) and airway tolerability should be incorporated into release and safety testing [66].

6.1.5. Topical/ophthalmic

Topical and ocular routes enable high local exposure with minimal systemic burden. EV eye drops improved corneal epithelial closure (~77 % vs 42 % control at 72 h) [67]; microneedle patches permit minimally invasive, controlled dermal delivery [68]. Reports should include surface residence/retention time, preservative compatibility, and dosing-frequency constraints [67,68].

Across all delivery routes, standardised reporting is essential for cross-study comparability. Studies should pre-specify dose in two orthogonal units (particles plus protein) and define sampling/assessment windows a priori. Route-specific exposure metrics must be captured: for IV delivery, early MPS uptake and circulating half-life; for local routes, target-site retention and rationale for dosing frequency; for intranasal administration, region-wise brain uptake with controls for pulmonary spill-over; for inhaled delivery, aerosol integrity and regional deposition; and for topical applications, surface residence time and preservative compatibility. Where relevant, matched safety laboratories—complement/coagulation for IV, respiratory tolerability for inhaled, ocular surface tolerability for ophthalmic—should be included alongside pre-specified comparability triggers for any engineering modifications [62–68].

6.2. EV engineering to enhance therapeutic potential

6.2.1. Surface modification and targeting

PEGylation can reduce opsonisation but may induce anti- PEG antibodies and accelerated blood clearance on repeat dosing [63]. Biomimetic cloaking (e.g., platelet-membrane coatings) has enhanced myocardial homing and macrophage-targeted delivery in myocardial infarction (MI) models [69]. Ligand-directed strategies (Lamp2b-rabies virus glycoprotein; cyclic arginine-glycine-aspartic acid (RGD)) increase uptake by neuronal or ischaemic tissues [70]. Magnetic guidance of superparamagnetic iron oxide nanoparticle-loaded MSC-EVs augments localisation in stroke and kidney-injury models [71,72]. Engineering reports should specify ligand identity and surface density, monitor immunogenicity (e.g., anti-PEG), describe any device-related safety mitigations, and outline comparability plans when surface features change [63–72].

6.2.2. Cargo loading and enhancement

Pre-loading (engineering parent cells) enriches EVs with desired miRNAs/proteins [73]. Post-loading (electroporation, sonication) can introduce exogenous payloads but may yield low efficiency and membrane perturbation [74]. Chemical conjugation and hybrid EV-lipid nanoparticles enable tunable pharmacokinetics and have improved messenger RNA (mRNA) delivery in tendon models [73,74]. EV-based clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) delivery has achieved preclinical gene knock-down with signs of reduced off-targets [75], though platforms remain early-stage and require biodistribution/off-target risk management; AI-guided formulation is being explored to optimise loading whilst preserving vesicle integrity [76]. Studies should document orthogonal loading QC (payload identity, integrity, and encapsulation), dose-normalised exposure, and bridging potency/safety data for each loading change [73–76].

6.2.3. Parental-cell programming

Engineering EV-producing cells can create inherently targeted vesicles (e.g., cardiosphere-derived cells presenting a cardiomyocyte-homing peptide) with improved cardiac uptake and function [72]. Preconditioning (hypoxia, cytokine licensing) rewires EV cargo and often boosts pro-angiogenic or immunomodulatory activity without post-isolation modification [18]. Upstream changes should trigger predefined comparability (identity plus potency plus pharmacokinetics/biodistribution) [18,72].

6.3. Biomaterial integration for controlled release

6.3.1. Matrix design

Hydrogels and scaffolds retain EVs at the target site, protect cargo, and enable controlled release. Natural matrices (ECM-derived) offer biocompatibility; synthetic polymers provide tunable mechanics; composites can balance both [77]. Affinity tethers (e.g., CP05-CD63) can immobilise EVs for on-demand release [77]. Manuscripts should address potential sequestration, trigger conditions for release, and sterilisation compatibility [77].

6.3.2. Release kinetics and stability

Cross-link density, mesh size and charge govern release; fibre-reinforced gels often produce biphasic (burst to sustained) profiles. Encapsulation can stabilise EVs against proteases and pH extremes; gelatin hydrogels preserved EV bioactivity over 28 days [77]. When comparing platforms, report t50 %/half-time release and link release kinetics to mechanism-aligned potency readouts [77].

6.4. Advanced manufacturing approaches

6.4.1. Scalable production

TFF concentrates EVs more gently and at higher yield than UC [16]. Three-dimensional microcarrier and hollow-fibre systems, integrated with TFF, can substantially increase production within closed, GMP-compatible workflows [16,17,60]. Renewable sources (immortalised lines, induced pluripotent stem cell-derived MSCs) can expand supply, though iMSC-EVs may differ functionally, necessitating comparability as platforms evolve [58]. Scale-up reports should align dose units across stages and trend potency longitudinally [16,17,58].

6.4.2. Process innovation and monitoring

Microfluidic devices (size-based, immuno-affinity, acoustic) enable high-purity separation and can integrate in-line analytics [76]. Continuous processing linking culture to TFF to polishing in a closed loop reduces handling and contamination [16]. Looking ahead, advanced process-analytical technologies and data-driven control schemes are likely to support real-time quality analytics in EV manufacturing, although EV-specific implementations remain at an exploratory stage. Before routine deployment, models should be cross-site validated with predefined action limits and demonstrated interpretability [76].

6.4.3. Regulatory considerations

MISEV2018 and MISEV2023 set foundational expectations for EV identity, heterogeneity reporting, dose quantification and QC [25,26]. Therapeutic EVs are typically regulated as biological medicinal products, requiring GMP and robust chemistry, manufacturing and controls (CMC) documentation [19]. Regulators increasingly emphasise comparability and mechanism-linked potency (e.g., T-cell suppression, macrophage polarisation, endothelial tube formation) as part of release [19,58]. Programmes using xenogenic sources should implement stringent provenance/impurity controls, and PEGylated products should include anti-PEG risk management [19,63].

6.5. Integration and future directions

The convergence of bioengineering, smart biomaterials, scalable bioprocesses, and clearer regulatory frameworks is propelling MSC-EVs toward translation. Early trials continue to expand safety data and guide dose/route selection [64], whilst engineering and depots improve

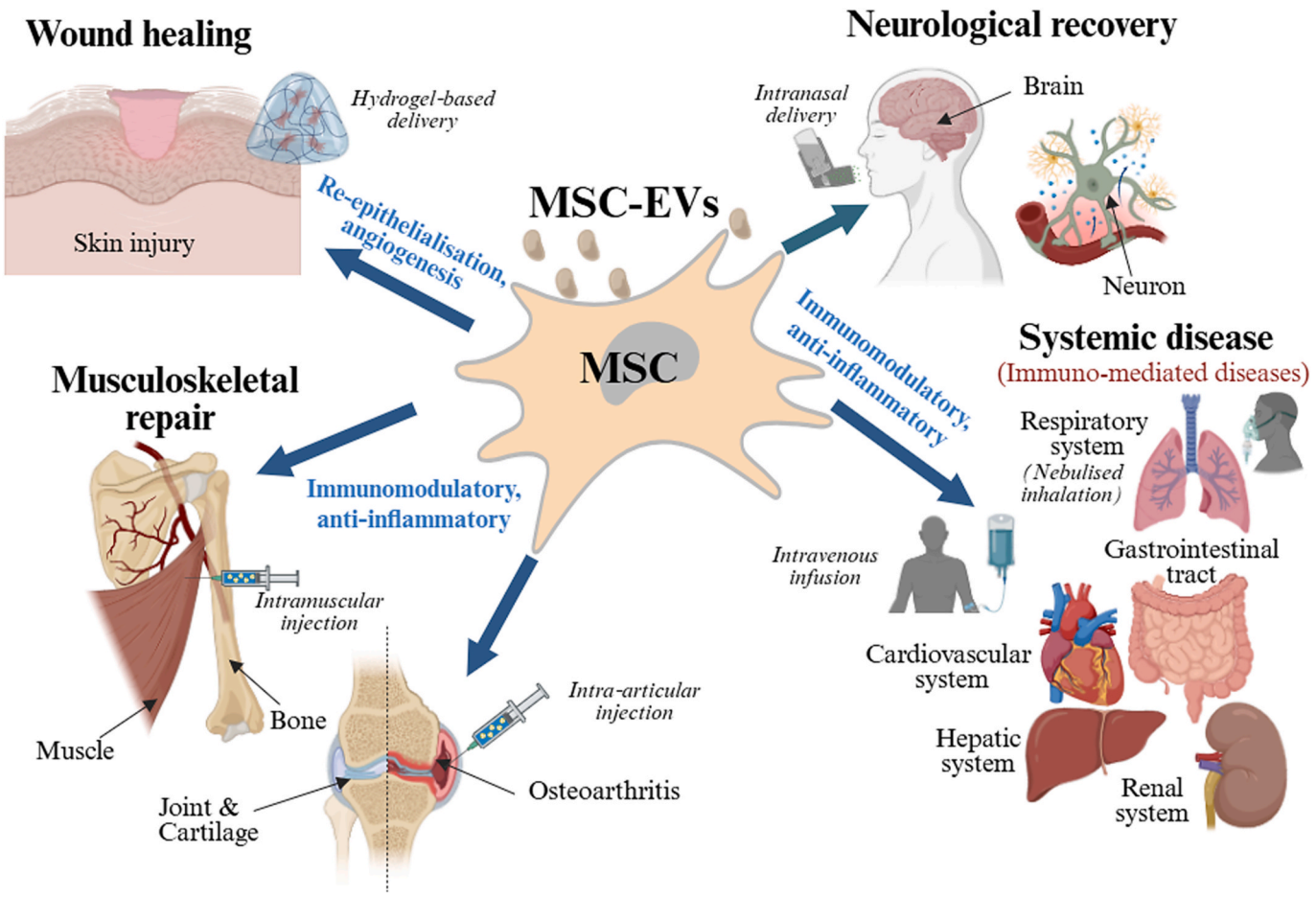

Fig. 2. Preclinical and emerging clinical therapeutic applications of MSC-EVs. Summary of major regenerative indications and organ systems where MSC-EVs show benefit in preclinical studies or early-phase clinical trials, including chronic wound healing, osteoarthritis and broader musculoskeletal repair, neurological recovery, and systemic immune-mediated diseases involving the respiratory, cardiovascular, hepatic, renal and gastrointestinal systems. Mechanistic targets, such as re-epithelialisation, angiogenesis, immunomodulatory and anti-inflammatory, together with representative delivery strategies, including hydrogel-based local delivery, intramuscular and intra-articular injection, intranasal administration, nebulised inhalation and intravenous infusion are illustrated. *The figure created with* BioRender.com*, accessed on November 20, 2025.*

exposure and effect size. Near-term priorities include head-to-head validation of delivery/engineering platforms, QC/potency standardisation, cost-effectiveness versus cell therapies, and real-world stability. Next-generation products may combine gene-editing payloads with AI-optimised manufacturing [76]. Ultimately, progress depends on exposure-response understanding, validated potency readouts, and pre-specified comparability plans that satisfy regulators and enable reproducible outcomes.

7. Clinical applications of MSC-derived EVs

MSC-EVs have emerged as a versatile therapeutic platform spanning cutaneous, musculoskeletal, neurological and systemic indications, with pleiotropic actions—immunomodulation, pro-angiogenic signalling, anti-apoptotic effects and extracellular matrix remodelling—that provide a mechanistic rationale for translation across organ systems [40,53,61]. Fig. 2 provides a cross-organ overview of current and emerging MSC-EV applications, delivery routes and typical readouts. Representative clinical and preclinical applications are summarised in Table 3, which also notes dose units and assessment windows for comparability.

7.1. Wound healing and skin regeneration

7.1.1. Mechanistic rationale

MSC-EVs orchestrate multiple phases of cutaneous wound repair via diverse bioactive cargo. They carry pro-regenerative microRNAs (miR-21, miR-126, miR-132, miR-210) and growth factors (VEGF, HGF, FGF-2, TGF-β1) that synergistically promote healing [39]. These factors polarise macrophages towards an M2 phenotype whilst dampening excessive M1 responses, establishing a pro-repair microenvironment [78]. EV-delivered miR-21 activates the PTEN/PI3K/AKT axis to enhance endothelial survival and neovascularisation [79]. EVs can also deliver developmental morphogens such as Wnt4 (from umbilical cord MSCs), activating β-catenin signalling to counter apoptosis and drive tissue regeneration [54]. In parallel, regenerative-medicine approaches for skin repair and their clinical use in Japan outline translational pathways and safety/feasibility considerations that align with EV-based cutaneous therapy [80,81].

7.1.2. Preclinical evidence

A 2024 systematic review and meta-analysis focused on diabetic wound models concluded that MSC-sEVs significantly accelerate closure and enhance angiogenesis, and that source and delivery route moderate effect sizes [82].

Subgroup analyses suggested that adipose-derived MSC-EVs and subcutaneous or local delivery may further optimise effects, although heterogeneity in isolation methods, dose units and regimens underscores the need for standardisation. Overall, preclinical gains have been observed across models, but dose, route and product characterisation remain important modifiers of effect size [82].

7.1.3. Bioengineering for enhanced delivery

Local delivery improves EV retention and efficacy. In diabetic wounds, gelatin methacryloyl hydrogels loaded with umbilical cord MSC-EVs outperformed free EVs, achieving faster closure, greater capillary density and more organised collagen [83]. Antimicrobial microneedle patches enabled controlled transdermal EV delivery, accelerating closure and supporting high-quality, appendage-forming repair in infected wounds [68]. Such platforms combine sustained release with EV protection in hostile wound environments. Notably, oxygen-releasing EV carriers have been developed to counteract hypoxia in chronic wounds—an exosome-coated oxygen nanobubble hydrogel significantly improved healing by enhancing angiogenesis, mitigating hypoxia and inhibiting inflammation in ischaemic wound beds [84].

7.1.4. Safety profile and clinical translation

Across early-phase human EV studies, short-term safety has generally been favourable and often comparable to placebo [85]. A Phase II, double-blind, randomised trial of IV bone-marrow MSC-EVs for COVID-19 ARDS (ExoFlo; NCT04493242) reported acceptable safety and an overall neutral 60-day mortality endpoint, with an exploratory signal in participants aged 18–65 years receiving two 15 mL doses [86]. For cutaneous indications, topical programmes are underway—for example, a recruiting trial of Wharton's jelly MSC-EVs gel for chronic diabetic foot ulcers (NCT06812637); peer-reviewed outcomes are not yet available (ClinicalTrials.gov, 2025). To date, published cutaneous studies have not reported EV-related immunogenic reactions or dose-limiting toxicities, but confirmation in adequately powered trials remains necessary.

7.1.5. Dermatological extensions: hair follicle regeneration

Beyond wound repair, regenerative-medicine strategies for hair growth and follicle regeneration illustrate adjacent cutaneous indications in which MSC secretomes, including EVs, and other cell-free approaches are actively being explored [80,81]. Together, these data support the rationale for developing targeted MSC-EV formulations for dermatological applications.

7.2. Musculoskeletal applications

7.2.1. Cartilage and joint repair

MSC-EVs carry chondroprotective microRNAs (e.g., miR-140-5p, miR-21) and growth factors that modulate TGF-β/bone morphogenetic protein (BMP) signalling, supporting cartilage homeostasis and suppressing inflammatory catabolism [87]. However, in a triple-blind, randomised, placebo-controlled trial in bilateral knee osteoarthritis, a single IA injection of placental MSC-EVs (5 mL per knee) was safe but not superior to placebo at 6 months on prespecified pain, function and MRI endpoints [88]. Preclinical syntheses indicate that EV source and repeat dosing influence cartilage outcomes, suggesting evaluation of multi-dose regimens and source-matched products in future trials [32].

7.2.2. Bone and soft tissue repair

EV-functionalised scaffolds (e.g., collagen/hydroxyapatite) enhance bone regeneration, increasing bone volume, neovascularisation and biomechanical strength compared with scaffolds alone [89]. In an Achilles tendon model, EVs from early-passage bone-marrow MSCs produced superior healing compared with EVs from senescent cells, with better collagen alignment and tensile strength [90], underscoring the value of source and culture optimisation. This suggests donor-cell ageing may diminish EV potency, supporting further evaluation of early-passage or rejuvenated sources [90].

7.3. Neurological applications

MSC-EVs have been reported to exert neuroprotective and neuroregenerative effects in multiple preclinical models, largely via transfer of miRNAs and proteins to neural and glial cells [40,65,71]. EV-delivered miR-133b, for example, down-regulates axonal growth inhibitors and enhances neurite outgrowth and plasticity after experimental stroke [46].

Intranasal delivery leverages olfactory and trigeminal pathways to bypass the blood–brain barrier; in rodent stroke and TBI models, intranasal MSC-EVs reduced neuroinflammation, preserved blood–brain barrier integrity and improved neurobehavioural recovery [65,71]. Similarly, in preclinical spinal cord injury models, systemically administered MSC EVs attenuated post-traumatic neuroinflammation and improved locomotor recovery [91]. A recent systematic review and meta-analysis of MSC-derived exosomes in spinal cord injury likewise reports significant improvements in locomotion, tissue preservation and neuroinflammatory markers across animal studies [92]. However, evidence that intranasal administration outperforms intravenous delivery is mixed, with route-dependent differences varying across models and endpoints [93,94].

Extending to neurodegeneration, a pilot, open-label safety study in amyotrophic lateral sclerosis (ALS) administered two 10-mL intravenous infusions of a bone-marrow MSC-EV investigational product 1 month apart (n = 10) and followed participants for 3 months; no treatment-related serious adverse events occurred and 30 % of subjects showed no decline on the ALS Functional Rating Scale–Revised during observation [95]. These preliminary, uncontrolled data broaden short-term intravenous safety experience into ALS and support progression to adequately powered, randomised trials with longer follow-up and route-aware pharmacology/biodistribution read-outs [95].

7.4. Systemic and emerging applications

7.4.1. Cardiovascular applications

In myocardial ischaemia, MSC-EVs reduce cardiomyocyte apoptosis and adverse remodelling in multiple preclinical models. Experimental targeted or inhaled strategies—for example nebulised MSC-EVs after myocardial infarction, platelet-mimetic coatings and integrin/RGD-based targeting—have been reported to improve myocardial localisation and functional recovery compared with non-targeted administration in animal studies [66,69,72]. Translation will depend on route- and dose-normalised exposure–response data and robust comparability between engineered and native EV products [66,69,72].

7.4.2. Respiratory applications

Randomised evidence now includes two controlled trials: (i) intravenous bone-marrow MSC-EVs (ExoFlo) in severe/critical COVID-19 respiratory failure (NCT04493242) demonstrated acceptable safety and overall neutral 60-day mortality, with an exploratory mortality/ventilator-free-days signal in participants aged 18–65 receiving two 15 mL doses [86]; and (ii) a double-blind RCT of human placental MSC-EVs as an adjunct to standard care in COVID-19-associated ARDS reported lower in-hospital mortality and improved respiratory indices versus placebo [96]. Open-label pilot studies of nebulised MSC-EVs have also suggested feasibility and improved oxygenation without EV-related adverse events [97,98]. Safety is encouraging overall, but confirmatory efficacy will require dose-ranging, route-specific trials with harmonised endpoints.

7.4.3. Renal and hepatic applications

Foundational studies show protection against acute kidney injury, with improved renal function and survival in cisplatin models after single-dose EV therapy [99]. Pilot human studies in chronic kidney disease reported feasibility of umbilical cord MSC-EV infusions with transient glomerular filtration rate (GFR) improvement and reduced proteinuria [100]. In liver disease, a 2025 meta-analysis concluded that MSC-EVs reduce collagen deposition and improve histological fibrosis scores across injury models, though clinical evidence remains limited [101]. Ongoing research is evaluating optimal delivery to these organs

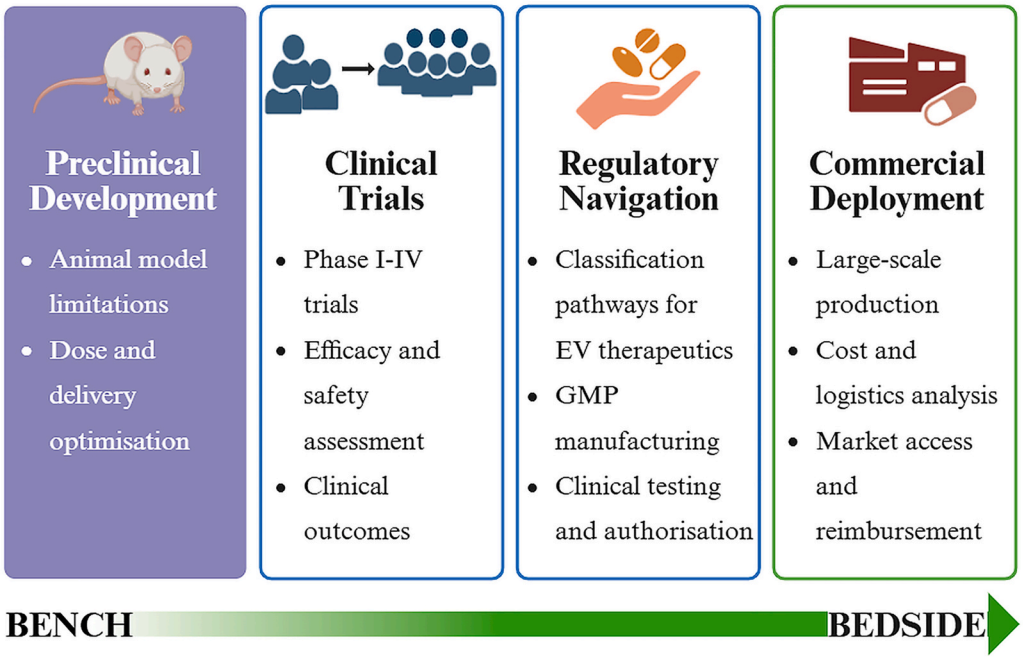

Fig. 3. Clinical translation roadmap for MSC-EV therapeutics. A schematic outlining the clinical translation continuum from bench to bedside, highlighting four critical domains: preclinical development (animal-model limitations and dose/delivery optimisation), clinical trials (Phase I–IV evaluation of efficacy, safety and clinical outcomes), regulatory navigation (classification pathways for EV therapeutics, GMP manufacturing and clinical testing/authorisation), and commercial deployment (large-scale production, cost and logistics analysis, and market access and reimbursement). The bench-to-bedside gradient bar at the bottom emphasises progression along this pathway. *The figure created with* BioRender.com*, accessed on November 20, 2025*.

(e.g., intrarenal injection or liver-targeted EV modification) to enhance local uptake and therapeutic impact [102].

7.4.4. Inflammatory bowel disease

In a murine model of ulcerative colitis, umbilical-cord MSC-EVs restored intestinal barrier integrity and reduced inflammation; EV-borne tumour necrosis factor-stimulated gene 6 (TSG-6) appeared critical for these effects, as depletion attenuated benefit and recombinant TSG-6 recapitulated key benefits [103]. These data support indication-specific cargo–mechanism alignment for future clinical development. A Phase I trial of MSC-EVs in medically refractory ulcerative colitis is underway (NCT05176366), and a first-in-human study of MSC-EVs for refractory perianal fistulas in Crohn's disease reported no serious adverse events and suggested an encouraging therapeutic effect [104].

7.4.5. Autoimmune and rheumatological disease

Whilst EV-specific trials in rheumatoid arthritis (RA) remain limited, MSC-based therapies for RA demonstrate clinically relevant immunomodulation and provide a translational scaffold for EV development in rheumatology (e.g., T-cell suppression, macrophage reprogramming, anti-inflammatory cytokine axes) [105]. Given that EVs recapitulate many MSC paracrine effects, rational EV design (dose and route; potency assays aligned to mechanism) warrants investigation in RA and related autoimmune indications.

8. Clinical translation and development considerations

The transition of MSC-EVs from experimental platforms to clinical-grade therapeutics is advancing but remains incomplete. From foundational insights into EV biology and intercellular communication [106] to early human signals—including a randomised controlled trial and a multicentre trial involving EVs in COVID-19 ARDS [96,97], progress has been steady yet heterogeneous. Growing preclinical evidence supports safety and efficacy, and early-phase clinical studies have established feasibility. To progress reliably towards clinical implementation, translation must integrate dose metrics and pharmacology, validated potency assays, scalable GMP manufacturing with comparability plans, and harmonised regulatory expectations (with adoption of MISEV2018/2023 reporting where applicable) [14,19,25,26]. Fig. 3 outlines this roadmap, from preclinical validation and potency development to GMP manufacturing and trial design.

8.1. Dosing units and pharmacology of MSC-EVs

MSC-EV dosing remains heterogeneous (particles, total protein/lipid, or "cell equivalents"). This variability complicates cross-study comparisons and exposure-response inference; accordingly, dosing should be reported in at least two orthogonal units (e.g., particles plus protein) and linked to a mechanism-reflective potency read-out, as detailed in Section 8.4. Per MISEV guidance, reports should also specify the measurement platform/model and analytical settings used for particle and protein quantification [14,25,26]. Pharmacology is route- and context-dependent: after systemic administration, rapid MPS clearance (liver/spleen) limits circulating exposure; local routes (e.g., IA, intranasal, inhalation) can increase target-tissue exposure.

Non-human primate and rodent studies show route-dependent biodistribution and disease-state modulation of uptake (e.g., greater lung accumulation during peak inflammation) [62]. Because MSC-EVs do not engraft, repeat dosing or sustained-release depots are often required to maintain pharmacodynamic activity. Table 3 includes "Dose metric & amount" and "Assessment window" to aid comparability across routes and indications.

8.1.1. Safety considerations (route-dependent)

Systemic administration warrants vigilance for: (i) thrombogenicity/coagulation (e.g., tissue factor-linked thrombin generation); (ii) complement activation and other immunogenicity risks with engineered coatings; and (iii) age/comorbidity effects on haemostasis and immune reactivity. For intravenous/systemic routes, comprehensive GLP safety

evaluation should include thrombin-generation assays (±tissue-factor activity), complement activation (e.g., CH50/AH50 or C3a/C5a split products), cytokine-release testing in human whole blood, and repeat-dose immunogenicity (anti-product antibodies; anti-PEG if PEGylated). Additionally, biodistribution/toxicokinetics with appropriate labelling, haematology/clinical chemistry, and organ histopathology in ≥2 species are essential. For local/depot routes, include local tolerance (e.g., joint/ocular surface), shedding to systemic circulation, and device/combination-product risks (if applicable). Assay acceptance criteria should match the intended clinical dose/window, with clear bridging rules when engineering changes are introduced (e.g., PEGylation, targeting ligands), in line with society statements and translational guidance [107–109].

8.2. Current clinical landscape

Early clinical trials span graft-versus-host disease, chronic cutaneous wounds, osteoarthritis, COVID-19-associated ARDS, and liver/kidney disease. Across these programmes, pooled evaluations of human EV studies indicate a generally favourable short-term safety profile, with adverse-event rates often comparable to placebo [85]. Signals of clinical benefit have been described in steroid-refractory GvHD (compassionate-use) [10], in COVID-19 ARDS trials involving EVs (including subgroup and double-blind findings) [96], and in pilot studies for chronic kidney disease [100]; however, most studies remain under-powered and require confirmation. Persistent heterogeneity in route, dose unit (particles, protein, cell equivalents), and dosing frequency underscores the need for harmonised reporting and potency-anchored dosing (see Section 8.4).

8.3. Manufacturing and regulatory challenges

Clinical translation is complicated by variability in MSC source, culture/priming, isolation methods, and dose units across programmes [19]. MISEV2018/2023 provide a foundation for identity, purity and characterisation [14,25,26], but regulators are increasingly emphasising validated potency assays, prospectively defined comparability plans, and clear release criteria alongside GMP controls [19]. Stability/logistics remain non-trivial; many EV products currently require −80 °C storage and are freeze-thaw sensitive. Lyophilisation strategies are being explored to enable refrigerated storage and reduce cold-chain burden [63]. EV-based therapeutics are typically developed within biological/advanced-therapy frameworks; early dialogue with regulators and pre-agreed comparability plans are therefore essential [19]. For improved yield and consistency, tangential-flow filtration with polishing (e.g., SEC or mixed-mode chromatography) is preferable to workflows relying solely on UC [16,17,19], ideally coupled to in-line/at-line analytics for identity, purity and potency. Comparability protocols must be invoked whenever bioreactors, media, or isolation steps change, with identity plus potency plus pharmacokinetics/biodistribution as the minimal bridging set.

8.4. Potency assays and product characterisation

A major hurdle is the lack of validated potency assays predictive of clinical efficacy. Candidate assays include T-cell proliferation suppression, macrophage polarisation, angiogenesis induction, and fibrosis inhibition [19]. For clinical development, a tiered approach distinguishing minimal lot-release requirements from extended development panels is recommended.

Minimal lot-release panel should include: (i) identity/characterisation per MISEV guidelines (CD9/CD63/CD81/TSG101/ALIX; ≥1 negative marker such as calnexin/GM130) [14,25,26]; (ii) dose metrics reported in particles plus protein with particle:protein trending; (iii) sterility/endotoxin, bioburden/nucleic acid testing as applicable; and (iv) one mechanism-of-action-aligned potency assay matched to indication (e.g., M1 to M2 polarisation for anti-inflammatory use; human umbilical vein endothelial cell (HUVEC) tube formation for pro-angiogenic use) with pre-specified acceptance criteria.

The extended development panel should add secondary functional assays (e.g., dendritic-cell maturation inhibition; myofibroblast suppression), route-aware pharmacokinetic/biodistribution labels, and stress/stability potency retention (e.g., post-lyophilisation). Any upstream or downstream process change (platform, media, isolation) should trigger comparability across identity, potency and pharmacokinetics/biodistribution.

Indication-aligned development assay sets include.

- Anti-inflammatory indications (e.g., ARDS, inflammatory bowel disease): Minimal—(a) CFSE-based CD3/CD28 T-cell proliferation suppression; (b) M1 to M2 macrophage polarisation (IL-6/IL-10 ratio, arginase-1/CD206). Extended—cytokine/chemokine multiplex; dendritic-cell maturation inhibition.
- Pro-angiogenic indications (e.g., diabetic foot ulcers/venous leg ulcers, ischaemic tissues): Minimal—(a) HUVEC tube formation (branch points/length); (b) scratch/wound-closure (migration index). Extended—endothelial nitric-oxide bioactivity; aortic-ring sprouting.
- Anti-fibrotic indications (e.g., liver or lung fibrosis): Minimal—(a) TGF-β-induced myofibroblast transdifferentiation (α-smooth muscle actin, phosphorylated SMAD2/3); (b) COL1A1/COL3A1 mRNA/protein. Extended—hydroxyproline; contractile-gel assays.

Product characterisation should report positive (CD9/CD63/CD81/TSG101) and at least one negative (e.g., calnexin/GM130) marker, size/concentration, sterility/endotoxin, and mechanism-relevant cargo attributes, with pre-specified acceptance criteria linked to potency [19,25,26].

8.5. Near-term priorities for clinical translation

- Standardise dosing and reporting. Report doses in ≥2 units (particles and protein; optionally cell equivalents) and include a pre-specified potency surrogate aligned to mechanism of action; state instrument/model and analytical settings for particle counting; define assessment windows a priori in line with expected pharmacokinetics/pharmacodynamics [14,19,25,26].
- Embed pharmacology into trial design. Incorporate route-specific biodistribution (intravenous vs local/intranasal/inhalation), optimise repeat-dose schedules, and plan exposure-response modelling [62,97].
- Strengthen safety evaluation. Implement comprehensive GLP panels for thrombogenicity/coagulation (e.g., tissue-factor activity), complement activation, and repeat-dose immunogenicity; stratify by age/comorbidity as outlined in Section 8.1.1 [19,109].
- Ensure comparability and CMC control. Pre-define change-control and bridging criteria for any process modifications (bioreactor, media, isolation). Use multi-attribute analytics to confirm batch consistency [16,19].
- Operationalise potency. Adopt indication-specific minimal vs extended assay sets (Section 8.4) with acceptance criteria tied to clinical endpoints [19,25,26].
- De-risk heterogeneity in trials. Consider multi-arm designs exploring dose, route, frequency with harmonised endpoints and biomarker panels [85,97].

Taken together, these priorities provide a practical pathway to reproducible, regulator-ready MSC-EV products.

9. Gaps, challenges, and future perspectives

Despite rapid progress, several scientific and translational

constraints still limit broad clinical adoption of MSC-EVs. Here, we distil critical knowledge gaps, technology bottlenecks, and near-term solutions, with explicit cross-walks to Table 4 (decision matrix) and the practical guidance captured in dual-metric dosing and exposure and minimal potency plus safety panels frameworks outlined in Section 8. The emphasis is on implementable steps that enable reproducible, regulator-ready products.

9.1. Critical knowledge gaps

9.1.1. Incomplete mechanistic understanding

How EVs sort bioactive cargo (RNAs, proteins, lipids), reach target cells, and elicit durable functional responses remains incompletely mapped [110]. Biogenesis, cargo loading and uptake are highly context-dependent; the same MSC source can yield compositionally distinct EVs under hypoxia versus normoxia or after inflammatory priming, complicating causal inference [25,26]. Near-term action: deploy perturbation-driven multi-omics (gain/loss of function cargo edits) coupled to mechanism-linked readouts (Section 8.4) to connect cargo modules to specific bioactivities and inform potency selection.

9.1.2. EV heterogeneity and functional attribution

Therapeutic preparations contain overlapping EV subtypes (small EVs, microvesicles, apoptotic bodies) with no single discriminatory marker [26,111]. It remains unclear whether a minority "high-value" subfraction or multi-cargo synergy drives efficacy [26]. Single-vesicle analytics (nano-flow, single-EV RNA, Raman) are promising but not yet standardised. If only a minority of vesicles carry critical cargo, bulk particle-count dosing may be inefficient, underscoring the need to identify and enrich potent subfractions. Near-term action: incorporate single-particle profiling of clinical lots in development studies and trend particle: protein with potency, as operationalised in Section 8 and Table 4 (Dose metrics).

9.1.3. Long-term and off-target effects

Long-term safety remains under-characterised, including risks of immune sensitisation, pro-coagulant or pro-inflammatory responses, and off-target accumulation in liver and spleen [85,112]. Whilst short-term signals are reassuring (Section 7), repeated dosing in older/comorbid populations is understudied [111]. Regulators call for extended monitoring and GLP thrombogenicity/complement packages pre-first-in-human [113]. Near-term action: implement the GLP safety panel specified in Section 8.1.1 and Table 4 (Safety), with repeat-dose designs and age/comorbidity strata.

9.1.4. Standardisation and reproducibility deficits

Isolation, quantification and potency assays are variably implemented across laboratories [111,114]. Even with identical source cells, cross-site reproducibility can be limited [111]. MISEV2018/2023 improved reporting expectations [25,26], but adoption remains uneven; meta-analyses continue to flag dose-unit inconsistency and reporting gaps [110,114]. Near-term action: prospectively embed MISEV2023 checklists and dual-metric dosing (Section 8) in standard operating procedures, and participate in inter-laboratory ring trials for potency (Table 4: Potency/Definitions).

9.2. Technological challenges

9.2.1. Scale-up without quality drift

Three-dimensional systems and tangential-flow filtration enable large-scale production, yet process variables (shear, oxygenation, media) can shift EV cargo and function [16,112]. Yield gains with 3D microcarriers (~140-fold) and HFB (~38-fold) demand head-to-head comparability with Quality-by-Design (QbD) controls [112]. Near-term action: lock a primary upstream/downstream platform and mandate side-by-side comparability (CQAs plus potency) for any change (Table 4).

9.2.2. Absence of validated potency assays

Particle/protein counts and marker panels do not predict efficacy [114–116]. Indication-aligned assays (e.g., macrophage polarisation, HUVEC tube formation, TGF-β myofibroblast suppression) are advancing [116], and multi-assay/omics-based classifiers may provide surrogates [117]. Regulators expect mechanism-of-action-aligned potency [19]. Near-term action: implement the minimal vs extended assay sets in Section 8.4 with pre-specified acceptance criteria, and enrol in ring trials (Table 4).

9.2.3. Stability, storage and formulation

−80 °C storage burdens cost and logistics; freeze-thaw can degrade cargo [111]. Product-specific lyophilisation (trehalose/sucrose) and depot matrices can preserve potency at 4 °C/ambient [118]. Near-term action: perform accelerated/real-time stability with post-stress potency and define release limits (Table 4).

9.2.4. Analytical characterisation and quality control

Platform-dependent discrepancies (e.g., nanoparticle tracking analysis vs flow) complicate size/concentration metrics [114]. Emerging single-particle tools deepen profiling but are not harmonised; reference EV materials and rapid functional QC are needed [26]. Near-term action: calibrate instruments to common standards, apply MISEV-conformant identity panels, and add a rapid bioactivity test to batch release (Table 4).

9.3. Emerging technologies and approaches

Bioengineering and targeting. Surface functionalisation (ligands/peptides), PEGylation, and magnetic guidance aim to improve tropism and half-life [119,120].

Cargo loading and gene therapy. Pre-/post-loading strategies enable drug/RNA delivery; EV-mediated CRISPR/Cas systems have achieved edits in vitro/in vivo [93,121–125].

"Off-the-shelf" sources. Immortalised and induced pluripotent stem cell-derived MSCs offer scale but require stringent controls for oncogenicity/residual pluripotency [60].

Al/ML Models can optimise culture, flag comparability deviations, and infer potency from omics, but require cross-platform validation and interpretability [112,117].

Near-term action: treat each engineering/analytics advance as a process change—invoke comparability triggers (identity plus potency plus pharmacokinetics/biodistribution), and extend safety panels where coatings/novel excipients are introduced (Table 4).

9.4. Roadmap for accelerating clinical translation

A practical, near-term roadmap centres on five linked actions.

1. Potency first. Establish validated, mechanism-of-action-aligned potency panels and move toward composite indices benchmarked to in vivo efficacy [19,116].
2. Dose normalisation. Harmonise dose metrics with dual-metric reporting (particles plus protein) and an accompanying functional read-out to enable exposure-response analyses [26,114]; see Section 8.
3. Comparability by design. Pre-define change-control and bridging criteria; verify batch equivalence using multi-attribute analytics and bioactivity (Table 4) [19,26].
4. Safety depth. Expand Good Laboratory Practice programmes (biodistribution, immunogenicity, thrombogenicity/complement) with repeat-dose designs and age/comorbidity strata [85,113]; see Section 8.1.1.

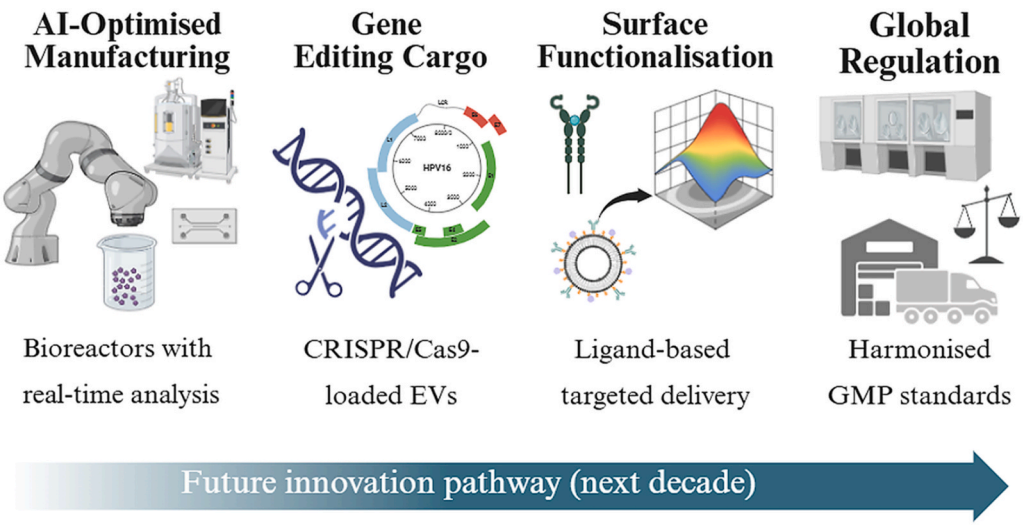

Fig. 4. Future directions in MSC-EV platform technologies and therapeutic applications. Conceptual diagram depicting future advances in MSC-EV development, including AI-optimised manufacturing with real-time bioreactor analytics, incorporation of gene-editing cargo (e.g., CRISPR/Cas9-loaded EVs), surface functionalisation to enable ligand-based targeted delivery, and global regulatory frameworks aimed at harmonised GMP standards. The horizontal arrow at the bottom indicates the projected innovation pathway over the next decade. *The figure created with* BioRender.com*, accessed on November 20, 2025.*

5. Field coordination. Intensify early regulatory dialogue; adopt reference EV materials, inter-laboratory proficiency testing, and shared clinical datasets to accelerate convergence on standards [26].

Together, these actions may facilitate progress toward the clinical translation of MSC-EV therapeutics.

10. Conclusions and prospects

Recent advances enable the bioengineering of MSC-EVs with customised cargo (e.g., overexpressed RNAs or proteins) and surface ligands to improve tissue targeting, with some platforms permitting controllable release. Nevertheless, major hurdles remain: validated potency assays are still lacking; product heterogeneity complicates cross-study comparability; regulatory classification and expectations vary across regions; and achieving scalable, GMP-compliant manufacturing is non-trivial. Whilst updated ISEVs guidance raises the bar for characterisation [26], genuine comparability will require mechanism-of-action-linked potency assays aligned with the intended route of administration. In parallel, practical enablers—closed, automated bioprocesses with TFF/SEC polishing and stabilisation strategies such as lyophilisation—are beginning to reduce logistical barriers and support clinical-grade supply [16,17,19,118].

Several patterns now recur across the literature. First, source matters: the tissue origin of MSCs strongly influences EV cargo and function, with therapeutic effects differing across bone marrow, adipose and umbilical cord sources; this variability argues for indication-matched sourcing and explicit linkage between source, hypothesised mechanism and potency read-outs. Second, delivery platforms matter: incorporating EVs into biomaterials such as hydrogels or scaffolds consistently improves localisation and retention versus bolus injections, often translating into larger effect sizes in vivo [79]. Third, priming enhances potency: hypoxia or cytokine licensing ('training') modulates EV yield and cargo, frequently augmenting immunomodulatory or regenerative activity [18]. Fourth, route shapes response: pharmacokinetic and biodistribution studies show that exposure and persistence are route-dependent, so dose units, potency assays and assessment windows should be tailored accordingly [62].

These lessons inform research and practice. For scientists, priorities include defining causal underpinnings of EV action, establishing MOA -aligned potency assays, and developing predictive biomarkers to guide product selection and patient stratification. Embedding exposure-response work—dual-metric dosing (particles plus protein), route-aware biodistribution, and regimen optimisation—into the preclinical-to-clinical continuum will accelerate learning and enable cross-programme comparability. For clinicians, near-term opportunities are strongest in localised indications—chronic wounds, osteoarthritis and neurotrauma—where early trials indicate safety and potential efficacy, local delivery is feasible, outcomes are measurable, and systemic exposure is limited [53,87,91]. Broader adoption will still depend on rigorous demonstration of durable efficacy, reproducible safety, and clear dosing guidance across routes.

From a manufacturing and regulatory perspective, efforts are converging on scalable, reproducible production. Automated, closed-system bioreactors (e.g., hollow-fibre designs) combined with TFF and SEC are already yielding clinical-grade batches at materially higher productivity [16,17]. To satisfy regulators and enable efficient scale-out/scale-up, developers should define CQAs early, implement in-line/at-line analytics for identity, purity and potency, and pre-specify comparability plans for any process change [19]. Such controls not only improve consistency but also reduce cost-of-goods, paving the way for distributed supply chains and stable formulations (e.g., lyophilised EV products) that can ease cold-chain constraints [118].

Looking ahead, the first approvals are most likely in niche, localised indications—for example, chronic ulcers or focal cartilage lesions—-where topical or IA administration enables high local exposure and objective endpoints. These early products may pave the way for next-generation engineered EVs, deliberately optimised for potency and precise targeting (e.g., tissue-specific homing ligands, enriched therapeutic RNAs, or even CRISPR/Cas payloads) [93,121–125]. Advances in lyophilisation may further stabilise EV products for ambient-temperature distribution and broaden access [118]. Notably, an IV bone marrow MSC-EV therapy for ARDS has advanced to a global Phase III evaluation (NCT05354141), although localised applications may face fewer delivery hurdles. Fig. 4 depicts this forward trajectory—from optimised production and rigorous analytics to indication-matched bioactivity and precision delivery—supported by AI-assisted bioprocessing, regulatory harmonisation and a maturing contract-manufacturing ecosystem.

Across indications, dual-metric dosing (particles plus protein) paired with MOA aligned potency surrogates should be the default reporting standard. A route-aware exposure-response lens—together with rational

depot/biomaterial use—can materially increase local exposure and effect size. Comparability anchored in CQAs and potency must be prespecified for any upstream (source, media, bioreactor) or downstream (TFF/SEC, lyophilisation) change. Stability and logistics (notably lyophilisation and closed processing) are near-term enablers of scalable, distributed supply. In the near term, localised indications with objective endpoints are the most plausible first approvals, with engineered/tissue-targeted EVs a credible next wave once potency and comparability are validated.

In sum, MSC-EVs are moving closer to clinical reality, but key challenges in potency, standardisation and regulatory alignment remain. With route-aware exposure–response, validated potency assays and prespecified comparability embedded into development, the field may be positioned to advance MSC-EVs toward safer, more scalable, off-the-shelf regenerative medicines.

Ethics approval and consent to participate

Not applicable.

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Declaration of competing interest

The authors have no conflicts of interest to declare for this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2025.101058.

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