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Mesenchymal Stromal Cells and Extracellular Vesicles in Regenerative Medicine: From Dynamic Cellular Signaling to Cell-Free Therapeutic Platforms

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Abstract

Mesenchymal stromal cells (MSCs) have been investigated extensively as therapeutic agents for tissue repair, inflammatory disease, and regenerative medicine. Their initial therapeutic rationale emphasized multipotent differentiation and replacement of damaged mesenchymal tissues. Accumulating evidence, however, indicates that durable engraftment and direct tissue replacement explain only a subset of observed effects. MSCs instead function as environmentally responsive stromal cells capable of modifying tissue repair through immunomodulatory, trophic, angiogenic, anti-apoptotic, antifibrotic, and extracellular-matrix regulatory signals. This mechanistic shift has focused increasing attention on the MSC secretome and particularly on extracellular vehicles (EVs), which transfer proteins, lipids, metabolites, and nucleic acids between cells and can reproduce selected biological activities of their parental cells. MSC-derived EVs (MSC-EVs) therefore offer a potential cell-free therapeutic strategy with distinct advantages in storage, formulation, engineering, and product standardization. Nevertheless, neither MSCs nor MSC-EVs constitute homogeneous therapeutic entities. Tissue source, donor characteristics, culture conditions, inflammatory or hypoxic priming, passage, senescence, isolation methods, storage, dose, route of administration, and recipient microenvironment can alter product composition and biological activity. Moreover, encouraging preclinical efficacy has not yet translated into uniformly convincing clinical benefit, and EV development remains limited by inconsistent nomenclature, separation and characterization methods, dose metrics, pharmacokinetic understanding, and validated mechanism-linked potency assays.

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This review integrates contemporary evidence on MSC biology, paracrine signaling, EV-mediated intercellular communication, regenerative mechanisms, tissue-specific applications, bioengineering, manufacturing, and clinical translation. We propose that future development should move beyond treating MSCs or "exosomes" as generic regenerative products toward indication-specific therapeutics defined by critical quality attributes, mechanism-linked potency, route-dependent exposure, and prospective clinical validation.

Keywords

Mesenchymal stromal cells; Extracellular vesicles; Regenerative medicine; Secretome; Exosomes; Paracrine signaling; Immunomodulation; Tissue repair; Cell-free therapy; Potency.

Introduction

Regenerative medicine seeks to restore tissue structure and function after injury, degeneration, inflammation, ischemia, or age-associated loss of repair capacity [1]. Although transplantation of differentiated or progenitor cells can in some settings provide direct cellular replacement, many regenerative processes depend equally on modification of the tissue environment in which endogenous repair occurs [2]. Inflammation, vascular integrity, extracellular-matrix composition, cellular senescence, metabolic stress, and communication among immune, stromal, endothelial, and tissue-resident progenitor cells collectively determine whether injury resolves through functional regeneration, incomplete repair, or fibrosis [3]. Therapeutic strategies capable of modifying this environment therefore represent a mechanistically distinct complement to direct cell replacement [4]. Mesenchymal stromal cells (MSCs) have become one of the most extensively investigated cellular platforms for this purpose. Historically isolated from bone marrow and subsequently identified in adipose tissue, umbilical cord, placenta, dental tissues, and other stromal compartments, MSC preparations are characterized operationally by plastic adherence, a characteristic surface-marker profile, and capacity for mesenchymal differentiation under defined in vitro conditions [5]. The International Society for Cell & Gene Therapy (ISCT) recommends the term mesenchymal stromal cells, rather than assuming "mesenchymal stem cells," unless stemness is demonstrated by rigorous functional criteria [6]. As such, specification of the tissue source is essential because MSC populations derived from different anatomical compartments are not biologically interchangeable. This distinction is more than semantic.

The original therapeutic concept of MSCs emphasized their ability to differentiate into osteogenic, chondrogenic, and adipogenic lineages and thereby directly replace injured tissue [7,8]. Although differentiation remains relevant for selected tissue-engineering applications, direct and durable engraftment frequently appears insufficient to account for the magnitude or diversity of biological effects reported after MSC administration [9]. Contemporary models instead emphasize MSC therapeutic function as responsive regulators capable of sensing inflammatory and injury-associated signals and modifying neighboring cells through soluble mediators, extracellular vesicles (EVs), extracellular matrix (ECM) interactions, and direct cell-cell communication [8,10,11]. Accumulating evidence indicates that paracrine signaling, including soluble and EV-mediated communication, contributes substantially to many MSC-associated therapeutic effects and, in numerous experimental settings, provides a more plausible explanation for observed biological activity than extensive donor-cell engraftment and differentiation [12-14]. This shift does not imply that MSCs function exclusively through secretion or that differentiation is therapeutically irrelevant. Rather, the relative contributions of transient paracrine signaling, direct cellular

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interactions, matrix remodeling, differentiation, and persistent engraftment are likely to vary according to product, route of administration, tissue environment, and indication. This model is consistent with the physiological identity of stromal cells as components of tissue microenvironments rather than simply reservoirs of replacement cells. MSC-like populations occupy perivascular and other stromal niches and participate in immune regulation, vascular support, matrix homeostasis, and communication with tissue-resident cells [15]. Therapeutically administered MSCs may therefore function as transient, environmentally responsive signaling units that alter the trajectory of tissue injury through immunomodulatory, trophic, angiogenic, anti-apoptotic, antioxidative, and antifibrotic mechanisms. Critically, these effects are context dependent, i.e., MSC phenotype and secretory activity change in response to inflammatory cytokines, hypoxia, ECM, three-dimensional culture, metabolic conditions, and other environmental stimuli. Recognition of this paracrine biology has generated intense interest in the MSC secretome and particularly in EVs.

EVs are membrane-enclosed particles released by cells that carry biologically active proteins, lipids, nucleic acids, and metabolites capable of altering recipient-cell phenotype [16]. MSC-derived EVs (MSC-EVs) can reproduce selected immunomodulatory and reparative activities associated with parental MSCs and potentially provide a more controllable cell-free therapeutic platform. At the same time, describing EVs simply as a safer or simpler substitute for MSCs risks repeating an earlier conceptual error: MSC-EVs are themselves heterogeneous biological products whose composition and activity depend on parental-cell identity, manufacturing environment, EV separation, formulation, dose, and route of administration. The terminology is particularly important. Under the current MISEV2023 framework, “extracellular vesicle” should generally be preferred to “exosome” unless endosomal biogenesis of the isolated particles has been demonstrated. Common size-based isolation methods do not by themselves establish exosomal origin, and EV preparations may contain heterogeneous vesicular and non-vesicular extracellular particles. Rigorous characterization and transparent reporting are therefore prerequisites for mechanistic interpretation and therapeutic development. This review examines MSCs and MSC-EVs as related but biologically and pharmaceutically distinct regenerative platforms. We first consider the evolution of MSC biology from differentiation-centered models toward dynamic stromal signaling, then examine soluble and EV-mediated mechanisms of tissue repair, determinants of MSC-EV composition and potency, and evidence across major regenerative applications. We subsequently evaluate the translational advantages and liabilities of viable MSC and MSC-EV products, with particular emphasis on manufacturing, characterization, dosing, biodistribution, potency, safety, and clinical evidence. Rather than assuming that cell-free products should replace viable MSCs, we propose an indication- and mechanism-dependent framework for determining when a responsive living-cell product, a defined secretome component, or an engineered EV therapeutic provides the most appropriate regenerative strategy.

MSCs as dynamic regulators of the regenerative microenvironment

The regenerative activity attributed to MSCs encompasses multiple mechanisms that are difficult to reconcile with a simple differentiation-and-replacement model. MSCs secrete cytokines, chemokines, growth factors, extracellular-matrix regulators, lipids, metabolites, and EVs that can alter immune-cell activation, endothelial behavior, cell survival, fibrosis, and endogenous progenitor responses. The relative contribution of each mechanism varies according to tissue source, injury state, delivery route, and host

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microenvironment. MSCs interact with innate and adaptive immune populations and can modify macrophage, monocyte, dendritic-cell, lymphocyte, and natural-killer-cell responses through soluble mediators, cell-associated signals, and EVs. Their immunomodulatory phenotype is environmentally responsive rather than constitutively fixed; inflammatory licensing, hypoxia, metabolic conditions, extracellular matrix, and other features of the recipient microenvironment can substantially alter MSC secretory and functional states. Consequently, therapeutic potency should be considered an interaction between product phenotype and disease context rather than an invariant property of an MSC preparation.

MSC-associated signaling can also influence endothelial survival and vascular remodeling, parenchymal-cell survival and oxidative stress, endogenous repair populations, and extracellular-matrix turnover. These activities provide plausible mechanisms for tissue protection and repair but should not automatically be interpreted as tissue regeneration. Enhanced angiogenesis may be beneficial in ischemic tissue yet undesirable in other contexts. For example, increased proliferation is relevant only when it produces appropriate functional tissue while reduced matrix deposition is not equivalent to restoration of normal extracellular-matrix architecture. These distinctions reinforce the need to define MSC activity according to indication-specific biological mechanisms and functional endpoints rather than a generic regenerative phenotype.

The MSC secretome: separating soluble and vesicular signaling

The MSC secretome comprises soluble proteins and peptides, lipids, metabolites, ECM-associated factors, and heterogeneous extracellular particles including EVs [17]. These components are not mechanistically equivalent. Soluble mediators may produce rapid receptor-mediated effects, whereas EVs can deliver complex molecular cargo into recipient cells and potentially produce more sustained phenotypic changes. As such, conditioned medium, concentrated secretome preparations, purified or enriched EV preparations, and defined EV subpopulations constitute different products which cannot be grouped simply as “MSC secretome therapy.” Rather, the composition, pharmacology, stability, manufacturing requirements, and regulatory characterization differ substantially.

EVs are membrane-bound extracellular particles released by essentially all cell types with cargo that can include membrane and cytosolic proteins, lipids, messenger RNA, microRNAs and other noncoding RNAs, metabolites, and other bioactive molecules [18]. Cargo composition reflects both cellular identity and physiological state, allowing EVs to function as context-dependent intercellular messengers [19]. EV preparations nevertheless encompass heterogeneous particle populations whose composition depends on cellular source, physiological state, culture conditions, and separation methodology [20]. This heterogeneity is not simply an analytical limitation; it may directly influence biological activity and therapeutic potency. A 2024 systematic review of 471 EV-related clinical trials found substantial methodological variability and limited consideration of EV subpopulations; only 11% incorporated EV subpopulations explicitly in experimental design [21]. Thus, increasing analytical resolution may reveal therapeutically distinct vesicle populations currently obscured by bulk measurements. The mechanistic attraction of MSC-EVs lies in their capacity to coordinate several processes required for regeneration rather than activate a single pathway [22].

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Biological activity observed with MSC-conditioned medium cannot be attributed to EVs unless vesicular and non-vesicular fractions have been experimentally separated and their respective activities tested using appropriate controls [23]. Conversely, activity observed in an EV-enriched preparation should not automatically be assigned to vesicular cargo because co-isolated proteins, lipoproteins, nucleic-acid complexes, or other extracellular particles may contribute to the measured effect [24].

Intercellular transfer of regulatory nucleic acids, proteins, lipids, and metabolites

EVs provide a protected mechanism through which multiple classes of biologically active molecules can be transferred between cells. MSC-derived EVs contain proteins, lipids, messenger RNAs, microRNAs, other noncoding RNAs, and metabolites, and uptake by recipient cells can alter transcriptional, signaling, metabolic, or functional states. This multimolecular cargo is one of the principal reasons EVs are attractive as regenerative therapeutics, but it also creates substantial challenges for mechanistic attribution. Consistent with MISEV2023 principles, detection or enrichment of a molecule within an EV preparation does not establish that the molecule is intravesicular, reaches the relevant recipient cell at a biologically active concentration, engages the proposed molecular target, or mediates the observed phenotype. This limitation is especially important for studies implicating individual microRNAs. A common experimental sequence consists of identifying an enriched microRNA by sequencing, predicting target pathways bioinformatically, documenting a concurrent change in one or more downstream proteins, and then attributing the therapeutic effect to that microRNA. Such evidence is useful for hypothesis generation but does not establish causality. A rigorous mechanistic claim requires demonstration that the candidate cargo is transferred to the relevant recipient cell, reaches a biologically effective intracellular concentration, engages the proposed molecular target, and is necessary or sufficient for the phenotype. This generally requires complementary loss-of-function and gain-of-function experiments, appropriate extracellular-vesicle controls, validation of target engagement, and rescue or reversal studies showing that manipulation of the proposed cargo changes the relevant biological effect. Where feasible, EV-depletion, cargo-depletion, uptake-inhibition, and rescue experiments provide complementary approaches for distinguishing EV-dependent activity from effects mediated by co-isolated or soluble components. Contemporary EV standards and translational reviews increasingly emphasize this distinction between compositional association and mechanism.

An additional consideration is that these mechanisms are unlikely to operate independently.

Immune modulation can alter angiogenesis and fibroblast behavior; vascular recovery can improve parenchymal survival; extracellular-matrix remodeling can affect immune-cell trafficking and progenitor-cell function; and EVs can influence several of these processes simultaneously. The biological appeal of MSC-derived EVs may therefore lie in coordinated modulation of an injury microenvironment rather than activation of a single pathway. At the same time, this pleiotropy makes product development more difficult because a broad catalogue of reported effects does not constitute a defined mechanism of action. Recent ISCT and regenerative-medicine frameworks consequently recommend identifying the context-specific active biological function of an MSC-EV product and linking manufacturing attributes to a mechanism-relevant potency assay early in development.

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MSC source is a product-defining variable

Bone marrow, adipose tissue, umbilical cord, placenta, dental tissues, and other sources yield stromal populations with overlapping but nonidentical transcriptional, metabolic, differentiation, and secretory phenotypes [25]. The same principle extends to their EVs. Tissue source should therefore be regarded as a critical material attribute, not merely a logistical choice. Recent systematic analyses reinforce source-dependent activity. For example, a 2025 meta-analysis of experimental bone-regeneration studies reported regenerative activity across MSC-EV sources but identified differing functional profiles among bone-marrow-, umbilical-cord-, and iPSC-derived MSC-EVs [26]. Although such preclinical comparisons should not be overinterpreted as clinical superiority, they demonstrate why EVs from different MSC sources should not be assumed to be interchangeable. Donor age, health, metabolic status, passage number, replicative senescence, oxygen tension, media composition, serum or platelet supplements, cell density, culture dimensionality, and harvest interval can further modify MSC phenotype and EV composition [27,28]. Thus, control or incorporation explicitly into product-development strategies is critical. Accordingly, efficacy demonstrated with EVs derived from one MSC source cannot be extrapolated without qualification to EVs derived from another source, even when both parental populations satisfy conventional MSC identity criteria. Tissue source, donor attributes, and manufacturing history should therefore be reported as integral components of product identity and considered explicitly in comparability assessments.

Priming and bioengineering: opportunity and additional complexity

One distinguishing feature of MSC biology is environmental responsiveness. Hypoxia, inflammatory cytokines, three-dimensional culture, biomaterials, pharmacologic stimuli, and other conditioning strategies can modify both MSC secretory activity and EV cargo. This provides an opportunity to generate therapeutically optimized products rather than relying on basal secretion. Recent literature describes hypoxic, cytokine, three-dimensional, and combinatorial priming strategies capable of enriching EV preparations for regenerative or immunomodulatory activity. However, priming converts culture conditions into product-defining manufacturing variables. A primed EV product cannot be assumed equivalent to an unprimed preparation from the same cell source. Exposure intensity, duration, timing, culture configuration, and harvest conditions require control, and any process-induced change should be linked to defined critical quality attributes and potency. EVs may also be engineered through modification of the parental MSC or post-isolation manipulation to alter cargo, surface properties, tissue targeting, or retention. Such strategies expand therapeutic possibilities but progressively transform EVs from naturally secreted biological products into engineered drug-delivery systems, with correspondingly greater requirements for characterization, biodistribution, off-target assessment, comparability, and safety. Enrichment of a desired biological activity does not establish an overall improvement in therapeutic profile. Priming may simultaneously alter unintended cargo, inflammatory activity, biodistribution, or other biological properties. Optimization should therefore evaluate the complete product phenotype rather than a single enhanced functional assay.

Preclinical efficacy across regenerative indications: breadth versus translatability

MSC-EVs have demonstrated biological activity across an unusually broad range of preclinical injury models, including musculoskeletal, cutaneous, cardiovascular, neurologic, renal, hepatic, and pulmonary

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disease. This breadth supports the biological plausibility of shared mechanisms involving inflammatory regulation, cytoprotection, vascular responses, and tissue remodeling, but it should not be interpreted as evidence that MSC-EVs constitute a single broadly effective therapeutic class. An umbrella review encompassing 47 meta-analyses across 27 disease categories reported favorable effects across multiple preclinical models but also identified high heterogeneity, frequent deficiencies in randomization and blinding, and evidence of publication bias [29]. These limitations substantially constrain inference regarding effect magnitude and clinical translatability

Musculoskeletal and skeletal repair

MSC and MSC-EV approaches have been investigated extensively in cartilage, bone, tendon, and osteoarthritis models. EV-mediated modulation of inflammation, chondrocyte survival, matrix homeostasis, angiogenesis, and osteogenic signaling provides biological plausibility for these applications. Meta-analytic preclinical evidence supports effects on bone regeneration, although source, dose, model, and administration route contribute substantial heterogeneity. The key translational challenge is moving from statistically positive animal studies to reproducible human benefit.

Cutaneous repair and chronic wounds

Chronic wounds provide a compelling application because inflammation, vascular insufficiency, impaired keratinocyte and fibroblast function, and abnormal matrix remodeling coexist within a locally accessible target. MSC-EVs have demonstrated effects on keratinocyte, fibroblast, and endothelial-cell behavior and angiogenic signaling in experimental wound models. Yet clinical evidence remains limited. A recent systematic review of published EV therapeutic studies found wound healing to be one of the more frequently investigated applications but concluded that controlled evidence remains insufficient to establish consistent efficacy. Local accessibility nevertheless makes wounds attractive for mechanism-linked dose optimization and biomaterial-assisted delivery.

Cardiovascular and ischemic injury

The combined angiogenic, cytoprotective, immunomodulatory, and antifibrotic activities attributed to MSC signaling provide a rationale for cardiovascular repair. However, myocardial regeneration requires distinguishing preservation of viable myocardium and remodeling of the post-injury environment from true generation of functional cardiomyocytes. MSC or MSC-EV therapies should not be described as myocardial regeneration when the demonstrated effect is predominantly cardio protection or remodeling.

Neurologic injury

MSC secretome and EV approaches are being investigated for stroke, traumatic injury, and neurodegenerative conditions. Proposed mechanisms include modulation of neuroinflammation, vascular responses, neuronal survival, and endogenous plasticity. The ability of EVs to interact with neural tissues and the potential for intranasal or engineered delivery are attractive, but biodistribution, target engagement, and dose-response relationships remain incompletely defined.

Renal, hepatic, pulmonary, and inflammatory injury

Preclinical literature reports MSC-EV effects across acute kidney injury, liver injury, pulmonary disease,

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and systemic inflammatory models. The breadth of these observations supports common mechanisms involving inflammatory resolution and tissue protection but simultaneously raises a translational concern: a therapeutic claimed to treat numerous biologically unrelated diseases through an undefined "regenerative" effect lacks a sufficiently precise mechanism of action. Clinical development should therefore prioritize indication-specific mechanistic hypotheses and potency assays.

The rationale for MSC-EVs is frequently presented as preservation of MSC paracrine activity without the complexities of administering viable cells. That proposition is plausible but incomplete.

Translational attribute	Viable MSC product	MSC-EV product
Biological behavior	Responsive living cell capable of changing phenotype after administration	Preformed biological particle population; cannot dynamically respond after administration
Mechanistic repertoire	Soluble secretion, EV release, cell-cell interaction, matrix interaction, environmental sensing	Cargo transfer and surface-mediated signaling
Product heterogeneity	Donor, source, passage, culture, viability, senescence and activation dependent	Parent-cell factors plus EV-production, separation and purification heterogeneity
Dynamic response to recipient environment	Potentially substantial	Limited to composition present at administration
Proliferative and biological safety considerations	MSCs have limited proliferative behavior but viable-cell risks require consideration	EVs are non-replicating, but safety remains dependent on molecular cargo, product purity, unintended biological activity, biodistribution, and contaminants or co-isolated extracellular components.
Storage/formulation	Viability-sensitive	Potentially more compatible with pharmaceutical formulation and storage
Dose definition	Cell number plus viability and potency	No universally validated dose metric; particle number, protein, active cargo or potency may differ
Biodistribution	Strongly affected by route; IV MSCs show substantial pulmonary first-pass distribution	Size- and route-dependent distribution; PK remains incompletely characterized
Potency	Mechanism-linked cellular assay required	Mechanism-linked EV assay required; particle number alone is insufficient
Engineering	Genetic or culture-based modification of cells	Parent-cell engineering and/or post-production EV modification possible
Principal theoretical advantage	Adaptive biological responsiveness	Cell-free standardization and engineering potential
Principal translational challenge	Product heterogeneity and inconsistent efficacy	Product definition, dose, purification, potency and pharmacology

The central distinction is therefore **adaptive versus predefined biology**. A viable MSC can sense its environment and alter its response after administration; an EV product delivers the biological information incorporated during manufacture. This may make MSCs advantageous when dynamic environmental responsiveness is required and EVs advantageous when a reproducible, defined biological signal can be manufactured prospectively.

Manufacturing and product characterization

Manufacturing is not merely a scale-up problem for MSC-EVs as it determines product identity. Cell source, culture medium, supplements, oxygen tension, confluence, bioreactor conditions, harvest interval, separation technique, concentration, purification, filtration, formulation, storage, and freeze-thaw exposure can all affect EV composition and function.

MISEV2023 provides the essential research framework for EV production, separation, characterization,

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and functional analysis and should form the methodological baseline for studies supporting therapeutic development. Clinical-grade development, however, requires additional CMC controls establishing reproducible critical quality attributes across manufacturing lots. A clinically meaningful product specification should address identity and composition, purity from process- and media-derived contaminants, particle concentration and size distribution, sterility, endotoxin and adventitious agents, stability, and functional potency. Where serum, platelet lysate, or other extracellular-particle-containing supplements are used, contamination with non-MSC-derived particles must be explicitly controlled. For MSC-EVs, the manufacturing process is inseparable from product definition because changes in upstream culture or downstream purification can alter both particle composition and biological activity. Process development should therefore establish relationships among critical material attributes, critical process parameters, critical quality attributes, and functional potency. Comparability after changes in cell source, media, culture format, bioreactor scale, separation technology, formulation, or storage should be demonstrated rather than inferred from similar particle concentration or size distribution. Analytical characterization should use complementary methods because no single assay establishes EV identity, purity, concentration, structural integrity, and biological function. Particle enumeration alone is particularly insufficient for demonstrating product equivalence or potency.

Dose, biodistribution, and pharmacology: an unresolved translational bottleneck

EV therapeutics currently lack a universally accepted biologically meaningful dose unit. Studies variously report particle number, total protein, source-cell equivalents, EV volume, or combinations thereof. These quantities are not necessarily interchangeable and may correlate poorly with active cargo or functional potency. Clinical-trial analyses confirm substantial heterogeneity in EV characterization and dosing. A 2025 analysis of 66 MSC-EV/exosome trials found major variation in dose units and suggested that effective exposure may be strongly route dependent. A contemporary translational framework has consequently proposed dual-metric characterization coupled to mechanism-aligned potency rather than reliance on particle count alone. This should be treated as a pharmacology problem. Route of administration determines exposure, biodistribution, clearance, cellular uptake, and target-organ delivery. A particle dose administered intravenously cannot be assumed pharmacologically equivalent to the same particle number delivered intraarticularly, intranasally, topically, or by inhalation. Future studies require explicit dose-exposure-response relationships rather than empirical dose selection. Detection of labeled EV-associated signal within an organ should not automatically be interpreted as intact-vesicle delivery or functional target engagement because labeling strategies may track dissociated label, membrane components, or degradation products. Pharmacokinetic and biodistribution studies should therefore distinguish, where technically feasible, circulating or tissue-associated signal from intact and biologically active EV exposure.

Potency should link product composition to mechanism of action

Perhaps the most important obstacle to MSC-EV translation is the absence of broadly validated potency strategies. Particle number, protein concentration, tetraspanin abundance, or presence of selected miRNAs establishes aspects of product characterization but does not establish therapeutic potency. Potency should instead be linked to the intended mechanism and indication. An EV product developed for inflammatory tissue injury may require a validated immunomodulatory assay; an angiogenic product may require endothelial functional activity; a cartilage product may require an assay linked to chondrocyte

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survival or matrix homeostasis. A potency assay need not reproduce every proposed biological effect of a pleiotropic product; rather, it should measure one or more activities sufficiently proximal to the proposed mechanism of action to discriminate biologically meaningful lot-to-lot variation. Where a single assay is inadequate, an orthogonal potency matrix combining complementary functional and molecular measures may be necessary, particularly when therapeutic activity is multimodal. This has become a central translational priority. The ISCT 2025 MSC-EV synthesis specifically identified definition of the context-specific active ingredient, alignment of manufacturing with critical quality attributes, and early incorporation of mechanism-linked potency assays as requirements for regulatory progress.

Clinical evidence: promise remains ahead of proof

The clinical maturity of MSCs and MSC-EVs differs substantially. MSCs have undergone extensive clinical investigation and generally demonstrate favorable safety, but efficacy across indications has been inconsistent. This history should temper assumptions that EVs will automatically translate more successfully simply because they capture selected MSC paracrine functions. MSC-EV clinical development remains early. A scoping review identified 73 registered cell-derived EV trials, with MSCs representing the predominant parental-cell source; studies were heterogeneous in design, characterization, and endpoints [30]. A recent systematic review of published therapeutic EV studies found only 25 eligible clinical studies, of which seven were controlled reporting on MSC-EVs had been administered to 494 patients [31]. Although administration was generally reported as tolerable, adverse-event reporting was incomplete and efficacy varied substantially by indication. The regulatory landscape has nevertheless evolved. The recent first U.S. FDA approval of an MSC therapy for pediatric steroid-refractory acute graft-versus-host disease demonstrates that an MSC product can achieve regulatory approval when manufacturing, product characterization, clinical indication, and efficacy evidence are sufficiently aligned. This precedent should not be generalized across MSC products or indications, but it underscores that translational success depends on development of a defined product for a defined clinical context rather than validation of MSCs as a generic therapeutic class.

The translational history of MSCs therefore provides an important caution for MSC-EV development. Extensive biological plausibility, a large preclinical literature, and favorable early safety are not sufficient substitutes for a reproducibly manufactured product, a defensible mechanism of action, a validated potency strategy, rational dose selection, and controlled evidence of clinical benefit. MSC-EV development should incorporate these requirements prospectively rather than attempting to resolve them after efficacy studies have begun.

A translational framework for regenerative development

Future development of MSC and MSC-EV therapeutics centers five prospective questions.

1. What biological defect is being targeted? The therapeutic objective should be specified as inflammatory resolution, cytoprotection, angiogenesis, antifibrotic remodeling, progenitor support, or another measurable process rather than generic “regeneration.”
2. Which product architecture is mechanistically necessary? Investigators should determine whether the indication requires the adaptive response of viable MSCs, a soluble secretome, a defined EV preparation, or an engineered EV product.

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3. What constitutes the active product and which attributes define lot comparability? Cell source, manufacturing conditions, EV population, purity, relevant active components, and critical quality attributes should be defined sufficiently to establish product identity and determine whether manufacturing changes or successive lots remain biologically comparable.
4. Which potency assay predicts relevant biological activity? Potency should be linked prospectively to mechanism and ultimately correlated with clinical response.
5. What exposure is required at the target tissue? Dose, route, biodistribution, persistence, and pharmacodynamic response must be integrated rather than optimized independently.

This framework shifts the field from asking whether "MSCs work" or "exosomes work" toward testing defined regenerative products against defined biological mechanisms.

Evidence gaps and priorities for the field

The principal limitation of the current evidence base is not simply insufficient study volume but insufficient comparability across studies. Differences in MSC source, donor characteristics, culture conditions, EV separation and purification, characterization, dose metrics, administration routes, disease models, and outcome definitions frequently prevent meaningful synthesis across nominally similar MSC-EV interventions. Moreover, mechanistic studies often rely on associative cargo profiling, preclinical efficacy studies remain vulnerable to inadequate randomization and blinding, and clinical studies are generally small and heterogeneous. An umbrella review of 47 meta-analyses across 27 disease categories illustrates this paradox: broad efficacy signals coexist with high statistical heterogeneity, recurrent risk of bias, and evidence of publication bias. The priority for the field should therefore shift from generating additional demonstrations that an MSC-EV preparation can produce biological effects toward establishing reproducibility, causal mechanism, product comparability, dose-response relationships, target engagement, and prospective clinical efficacy. MISEV2023 provides an essential framework for rigorous EV research, but therapeutic development additionally requires pharmaceutical-quality product definition, validated potency, manufacturing controls, and clinically relevant pharmacology.

Conclusions

The scientific basis for MSC-based regenerative therapy has shifted from a predominantly cell-replacement model toward recognition of MSCs as environmentally responsive stromal regulators whose effects can be mediated through soluble and EV-associated signaling. This evolution provides a rationale for MSC-EVs as cell-free therapeutic candidates but does not establish them as simpler or inherently superior substitutes for viable MSCs. Instead, EV development relocates the major translational challenges to product identity, heterogeneity, purification, dose, pharmacology, potency, and manufacturing control.

The relevant question is therefore not whether MSCs or MSC-EVs are broadly regenerative, but which product architecture can reproducibly modify a defined disease mechanism with an acceptable benefit-risk profile. Viable MSC products may offer a theoretical advantage when therapeutic activity requires dynamic environmental sensing, whereas MSC-EVs may be preferable when the relevant biological activity can be prospectively defined, manufactured, characterized, and delivered as a reproducible product. Progress toward clinical translation will depend on causal mechanistic evidence, mechanism-linked

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potency, controlled manufacturing, route-appropriate pharmacology, and adequately powered clinical studies rather than continued accumulation of broadly positive preclinical observations.

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