pharmaceuticals

MDPI

Systematic Review

Lyophilized Mesenchymal Stromal Cell-Derived Products in Regenerative Medicine: A Systematic Review of Biological Activity, Stability, Safety, and Therapeutic Effects

Bolat Sadykov [1], Abay Baigenzhin [2], Anastassiya Ganina [2], Madina Karimova [2], Aizhan Akhayeva [2], Anuar Zhunussov [2], Batyrbek Assembekov [1] and Ildar Fakhradiyev [1,3,*]

[1] Department of Medicine, S.D. Asfendiyarov Kazakh National Medical University, Almaty 050000, Kazakhstan; assembekov.b@kaznmu.kz (B.A.)
[2] National Scientific Medical Center, Astana 010009, Kazakhstan
[3] College of Medicine, Korea University, Seoul 02841, Republic of Korea
* Correspondence: fakhradiyev.i@kaznmu.kz; Tel.: +7-707-500-1190

Abstract

Background/Objectives: Lyophilization may facilitate storage and distribution of mesenchymal stromal cell (MSC)-derived products. This systematic review assessed biological activity, storage stability, safety, and therapeutic effects of lyophilized MSC-derived products in regenerative medicine. **Methods:** PubMed, Scopus, and Web of Science Core Collection were searched through 28 August 2026. Eligible English-language original studies included in vivo animal or human therapeutic evaluations. The review was registered in PROSPERO (CRD420261426429) and reported following PRISMA 2020. Two reviewers independently selected studies, extracted data, and assessed risk of bias using SYRCLE, RoB 2, or ROBINS-I according to study design. Heterogeneity precluded meta-analysis; findings were synthesized narratively. **Results:** Thirty-nine studies were included from 884 records; only one contained a human interventional component. Products included conditioned medium, secretome, extracellular vesicles, and whole-cell or synthetic preparations. Sixteen studies evaluated biological activity or stability: 12 directly compared lyophilized and fresh or non-lyophilized products, whereas four assessed post-lyophilization activity without a fresh or non-lyophilized comparator. Retention depended on product formulation and processing conditions, and storage-stability evidence was limited. Twelve studies provided safety evidence; no major systemic toxicity signal emerged, although concentration-dependent cytotoxicity in vitro and transient local reactions occurred. Most studies reported improvement in at least one regenerative or functional outcome, with neutral or mixed findings in some applications. Incomplete reporting of randomization and blinding limited confidence in the evidence. **Conclusions:** Some lyophilized MSC-derived products retain biological properties and exhibit therapeutic activity, predominantly in preclinical models. Evidence is insufficient to establish clinical efficacy or long-term safety. Translation requires standardized manufacturing, validated potency and storage-stability testing, systematic safety assessment, and well-designed human studies.

Keywords: mesenchymal stromal cells; lyophilization; secretome; conditioned medium; extracellular vesicles; regenerative medicine; systematic review

Check for updates

Academic Editor: Leonidas A. Phylactou

Received: 13 October 2026
Revised: 13 November 2026
Accepted: 16 November 2026
Published: 18 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Mesenchymal stromal cells (MSCs) are widely investigated in regenerative medicine, and a substantial component of their therapeutic activity is thought to be mediated by

paracrine signaling rather than sustained engraftment and direct differentiation within injured tissues [1]. MSCs release soluble proteins, cytokines, growth factors, lipids, nucleic acids, and extracellular vesicles (EVs) that constitute the MSC secretome and can be investigated as conditioned medium, secretome preparations, or isolated extracellular vesicles [1,2]. Recognition of the paracrine activity of MSCs has increased interest in MSC-derived cell-free therapeutics, which may retain regenerative and immunomodulatory functions of parental cells while allowing the therapeutic product to be characterized, formulated, and stored without viable-cell administration [2].

Despite their therapeutic potential, the clinical translation of MSC-derived cell-free products requires preservation strategies that maintain product integrity and biological activity during storage, transport, and use. Conventional frozen storage imposes substantial cold-chain requirements, whereas lyophilization can convert biological preparations into solid formulations that are easier to store and distribute and may facilitate longer-term preservation and point-of-care availability [3]. However, freeze-drying is not a biologically neutral process: freezing, dehydration, storage, and reconstitution can alter the structural and molecular characteristics of extracellular vesicles and other bioactive components, with consequences for their functional activity [4]. Experimental studies of MSC-derived secretome products further show that successful lyophilization depends on formulation and process conditions, including the choice of lyoprotectant, and that biological activity cannot be assumed to be preserved simply because a product has been freeze-dried [5].

Lyophilization is not a standardized intervention that simply converts an otherwise unchanged MSC-derived product from a liquid or frozen state into a dry formulation.

The properties of the final product depend on the lyoprotectants and excipients used, freezing and drying parameters, residual moisture, reconstitution and storage conditions, as well as upstream factors such as MSC source, product type, and isolation or concentration procedures [5,6]. Incorporation into carriers, hydrogels, or scaffolds may further modify product characteristics and therapeutic performance [7]. Biological activity observed after lyophilization does not, by itself, demonstrate retention of activity through the lyophilization process. This requires comparison with an appropriate fresh or non-lyophilized product. Similarly, reported storage conditions do not demonstrate storage stability unless relevant product characteristics or biological activity have been evaluated over time. These distinctions are essential when interpreting the biological and translational evidence for lyophilized MSC-derived therapeutics.

Studies of lyophilized MSC-derived therapeutic products now span different product types, formulations, regenerative indications, and experimental models, but the evidence remains predominantly preclinical.

Previous reviews have examined MSC secretome and extracellular vesicles in regenerative medicine, extracellular vesicle preservation and storage, freeze-dried MSC preparations, scaffold-based secretome delivery [3,4,7,8], and indication-specific applications such as wound healing and bone regeneration [9,10]. However, these reviews have generally focused on individual product classes, preservation strategies, delivery systems, or clinical indications rather than systematically integrating evidence across the spectrum of lyophilized MSC-derived products. As a result, evidence on biological preservation, storage stability, safety, and therapeutic effects has not been systematically evaluated across these product types within a common framework. This systematic review examines the biological activity, stability, safety, and therapeutic effects of different lyophilized MSC-derived products, with particular attention to the quality and limitations of the available evidence.

It remains unclear how consistently lyophilized MSC-derived products retain their biological properties, what evidence supports their stability and safety, and whether biological activity after lyophilization translates into therapeutic effects in vivo or clinically. This sys-

tematic review therefore aimed to evaluate and integrate the available preclinical and clinical evidence on the biological activity and stability, safety, and therapeutic effects of lyophilized MSC-derived products used in regenerative medicine. Particular attention was given to distinguishing direct evidence of preservation through lyophilization from post-lyophilization potency, formal storage-stability evidence from reported storage conditions, and effects attributable to lyophilization from those of the complete therapeutic formulation.

2. Materials and Methods

2.1. Protocol and Reporting Standards

This systematic review was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD420261426429) on 17 June 2026. The review was designed to evaluate the biological activity and stability, safety, and therapeutic effects of lyophilized MSC-derived products used in regenerative medicine across preclinical and clinical studies. A separate full review protocol was not prepared. Prespecified review information was documented in the PROSPERO registration record.

The review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [11]. Study identification, screening, eligibility assessment, and inclusion were documented using a PRISMA flow diagram. Methodological refinements made after registration are described below.

2.2. Eligibility Criteria

Studies were eligible if they evaluated a lyophilized MSC-derived product in an in vivo animal study or in human participants and addressed a therapeutic or regenerative medicine application. Eligible animal studies included experimental models of disease, injury, tissue repair, or regeneration, as well as therapeutic studies in veterinary patients with naturally occurring disease. Human studies were eligible irrespective of participants' age, sex, ethnicity, or disease condition, provided that a lyophilized MSC-derived product was administered for therapeutic or regenerative purposes.

Eligible interventions included lyophilized MSC-derived products from any tissue source. Products of interest included conditioned medium, secretome, extracellular vesicles, exosomes, apoptotic vesicles, whole-cell lyophilized MSC preparations, and other MSC-derived biological products. These product categories were included within a single review because the primary focus was the application of lyophilization to MSC-derived therapeutic preparations, rather than the comparative efficacy of biologically equivalent interventions. Their differences in composition, mechanisms of action, manufacturing requirements, and stability characteristics were acknowledged, and findings were interpreted according to product type without assuming interchangeability or pooling therapeutic effects across categories. For the purposes of eligibility assessment, composite formulations were considered eligible when the MSC-derived component itself had undergone lyophilization, either before incorporation into a carrier or as part of the final therapeutic formulation. Studies in which lyophilization applied only to an unrelated carrier or scaffold, while the MSC-derived component itself remained non-lyophilized, were not eligible. No restrictions were applied according to disease indication, organ system, route of administration, dose, treatment duration, or follow-up period. No predefined minimum characterization criteria for MSCs or their derived products were applied as eligibility requirements. Available characterization data were extracted and considered when interpreting the findings.

Both comparative and non-comparative studies were eligible. Acceptable comparators included untreated or vehicle controls, placebo or usual-care groups, active treatments, fresh or non-lyophilized MSC-derived products, alternative lyophilized formula-

tions, or other study-specific controls. Randomized and non-randomized study designs were considered eligible.

Studies were required to report at least one outcome relevant to biological activity and stability, safety, or therapeutic effects. Biological activity and stability outcomes included preservation of structural, physicochemical, molecular, biochemical, histological, or functional properties after lyophilization, post-lyophilization biological potency, and stability during storage. Safety outcomes included adverse events, serious adverse events, mortality, toxicity, immunogenicity, treatment-related complications, or other safety findings. Therapeutic effects included disease- or indication-specific measures of tissue repair or regeneration, functional recovery, clinical improvement, biomarker changes, imaging outcomes, or other efficacy endpoints reported by the study authors.

Studies were excluded if they were exclusively in vitro without an eligible in vivo animal or human component, evaluated only non-lyophilized MSC-derived products, did not involve an eligible therapeutic or regenerative application as defined above, or did not report original research findings. Reviews, editorials, protocols, and conference abstracts without sufficient original data were excluded. Only peer-reviewed original research articles published in English were considered, with no publication-date restriction.

2.3. Information Sources and Search Strategy

A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science Core Collection. Searches were performed without publication-date restrictions or language filters. However, eligibility was restricted to English-language full-text publications in accordance with the prospectively registered review criteria. The search strategy was designed to identify studies evaluating lyophilized MSC-derived products and consisted of four complementary search branches. These targeted (1) MSC-derived secretome or conditioned medium, (2) extracellular vesicles, exosomes, microvesicles, or apoptotic vesicles, (3) whole MSC preparations, and (4) other MSC-derived products, including lysates, membranes, and microparticles. Each branch combined terms for MSCs or MSC-derived products with terms related to lyophilization or freeze-drying. Database-specific syntax was adapted to the search functionality of each source. No terms related to specific diseases, regenerative indications, safety, efficacy, or animal or human study design were included to maintain broad search sensitivity.

The complete database-specific search strategies are provided in Supplementary Table S1. The final database searches were conducted on 28 August 2026. The searches identified 192 records in PubMed, 412 in Scopus, and 280 in Web of Science Core Collection, yielding 884 records before deduplication.

The prospectively registered PROSPERO record specified MEDLINE/PubMed as the planned bibliographic source. During the study identification stage, the search was expanded to include Scopus and Web of Science Core Collection to improve coverage and reduce the likelihood of missing relevant studies. This represented an expansion of the registered search strategy. No changes were made to the review question or the principal eligibility framework.

2.4. Study Selection

Records retrieved from the three databases were combined into a single dataset. Duplicate records were identified using available PubMed identifiers, digital object identifiers, and normalized article titles. Following deduplication, all unique records underwent title and abstract screening against the predefined eligibility criteria. Records were retained for full-text assessment when they appeared potentially eligible or when eligibility could not be determined reliably from the title and abstract alone.

Full-text reports of potentially eligible records were assessed against all eligibility criteria, including whether the MSC-derived component itself, or a therapeutic formulation containing that component, had undergone lyophilization, whether an eligible in vivo animal or human component was present, and whether the study represented a therapeutic or regenerative medicine application. Studies in which a lyophilized MSC-derived product was used only for in vitro or ex vivo manipulation and was not part of the therapeutic intervention evaluated in vivo were excluded.

Title-and-abstract screening and full-text eligibility assessment were performed independently by two reviewers. Disagreements at either stage were resolved through discussion until consensus was reached. The primary reason for each exclusion at the full-text stage was recorded.

2.5. Data Extraction and Data Items

Two reviewers independently extracted data from the full-text reports using a predefined structured extraction framework. The resulting datasets were compared, and discrepancies or unclear items were resolved through re-examination of the source publication and discussion between the reviewers until consensus was reached.

Data were extracted across five main domains. These comprised (1) study characteristics, including author, year of publication, country, study design, experimental or clinical model, sample size, and follow-up duration, (2) characteristics of the MSC source and derived product, including tissue source, product type, and relevant preparation or characterization details, (3) lyophilization and formulation characteristics, including whether lyophilization was applied directly to the MSC-derived product or to a composite formulation containing it, the use of lyoprotectants or other excipients, carrier or delivery system, reconstitution procedure, and storage conditions and duration, (4) intervention characteristics, including dose or concentration, route and frequency of administration, treatment schedule, and comparator, and (5) outcome data related to post-lyophilization biological activity and stability, safety, and therapeutic effects.

For animal studies, sample size was extracted as the number of animals contributing to the relevant in vivo experiment whenever this could be determined from the report. Where studies contained multiple experimental components, only animals contributing to eligible in vivo comparisons were counted. In studies containing both preclinical and human components, sample sizes were recorded separately for each component where applicable. When the number of animals contributing to a specific analysis could not be determined unambiguously, it was recorded as not reported rather than inferred.

For biological activity and stability, extracted data included structural and physicochemical characteristics, molecular or biochemical composition, biological activity after lyophilization, comparisons with fresh or non-lyophilized preparations, and storage conditions and stability assessments. Safety data included mortality, adverse events, serious adverse events, local or systemic toxicity, laboratory abnormalities, immunogenic or inflammatory reactions, treatment-related complications, and other safety findings reported by the study authors. Therapeutic effects data included study-specific measures of tissue repair or regeneration, functional recovery, histological or imaging outcomes, biomarkers, organ function, and clinical outcomes.

Information that was not reported or could not be determined unambiguously from the published article was recorded as not reported (NR) rather than inferred. Study authors were not contacted to obtain missing or additional information, in accordance with the prospectively registered review record.

2.6. Outcome Definitions

Outcomes were organized into three domains: (1) biological activity and stability after lyophilization, (2) safety, and (3) therapeutic effects.

Biological activity and stability included structural, physicochemical, molecular, biochemical, and functional properties of lyophilized MSC-derived products. Direct evidence of retention after lyophilization was defined as a comparison of the lyophilized product with an appropriate fresh or non-lyophilized counterpart for the same structural, molecular, physicochemical, or functional property. Biological activity demonstrated after lyophilization without an appropriate fresh or non-lyophilized comparator was classified as post-lyophilization potency rather than direct evidence of retention. Storage stability was considered formally assessed only when relevant physicochemical, molecular, or functional properties were evaluated after a defined storage period, with an appropriate baseline or comparator permitting assessment of change over time. Reported storage conditions or duration alone were not considered evidence of storage stability.

Safety outcomes included adverse events, serious adverse events, mortality, local or systemic toxicity, laboratory abnormalities, immunogenic or inflammatory reactions assessed in a safety context, treatment-related complications, and other explicitly reported safety findings. Biomarkers measured solely as indicators of therapeutic response or disease activity were not classified as safety outcomes unless the study authors explicitly evaluated them in a safety or toxicity context.

Therapeutic effects comprised in vivo or clinical outcomes reflecting disease- or indication-specific therapeutic effects, including tissue repair or regeneration, functional recovery, histological or structural improvement, biomarker changes, imaging outcomes, organ function, and clinical outcomes. When a lyophilized MSC-derived product was evaluated as part of a composite formulation, therapeutic effects were attributed to the complete intervention rather than to the lyophilized component or the lyophilization process alone, unless the study design permitted these effects to be distinguished.

2.7. Risk-of-Bias Assessment

Risk of bias was assessed using design-specific tools according to the methodological characteristics of the included studies. Experimental animal studies were assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk-of-bias tool [12]. Randomized interventional studies in human participants or veterinary patients were assessed using the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) [13], whereas non-randomized interventional studies were assessed using the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) tool [14]. For studies containing more than one eligible study component with different designs, risk of bias was assessed separately for each component using the corresponding design-specific tool. SYRCLE was applied to experimental animal-study components irrespective of whether random allocation was explicitly reported, as it was used to assess potential sources of bias in the design and conduct of animal experiments rather than to assume that all experiments were randomized. Where randomization or other methodological safeguards were not adequately described, the corresponding domains were judged based on the available information. In vitro experiments conducted within otherwise eligible studies were considered as supporting evidence for biological activity, stability, or safety, but were not assigned separate risk-of-bias assessments using these tools, which were not designed for in vitro investigations.

For SYRCLE assessments, the domains considered included sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers and investigators, random outcome assessment, blinding of outcome assessors, incomplete

outcome data, selective outcome reporting, and other potential sources of bias. For RoB 2, risk of bias was evaluated across the five standard domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. For ROBINS-I, the domains considered were bias due to confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result. RoB 2 assessments focused on the principal therapeutic effects outcome of each randomized study component, whereas the ROBINS-I assessment focused on the principal safety/tolerability inference.

For each assessment, the relevant study component, outcome, and time point were specified. Domain-level judgements were based on information reported in the source publication. Overall judgements for RoB 2 and ROBINS-I were assigned according to the respective decision rules. No numerical or composite overall SYRCLE score was calculated. When methodological information was insufficiently reported, judgements were based on the available information rather than on the assumption that an unreported methodological safeguard had been implemented.

The PROSPERO record specified use of the original Cochrane risk-of-bias tool (RoB 1) and assessment by one reviewer with checking by a second reviewer. Design-specific tools were subsequently used because the included evidence comprised experimental animal studies, randomized veterinary and human studies, and a non-randomized veterinary study. Two reviewers independently performed all risk-of-bias assessments, compared their judgements, and resolved disagreements through discussion until consensus was reached. These changes did not affect study eligibility or the registered review outcomes.

2.8. Data Synthesis and Analysis

Given the heterogeneity in MSC sources, product types, lyophilization and formulation procedures, study models, comparators, and outcome measures, the evidence was synthesized using a structured narrative approach. Studies were organized according to study design and model, MSC source and product type, lyophilization and formulation characteristics, and three outcome domains: biological activity and stability after lyophilization, safety, and therapeutic effects.

Study characteristics and intervention-related variables were summarized at the study level. Categorical characteristics were summarized as numbers of studies. Variables such as dose, treatment duration, storage duration, and follow-up were reported descriptively when differences in definitions, units, or experimental context precluded meaningful aggregation. Findings were tabulated to allow comparison across studies while preserving the experimental or clinical context in which each outcome was evaluated.

For biological activity and stability, synthesis distinguished direct evidence of retention after lyophilization from post-lyophilization potency and formal storage-stability evidence. Safety findings were synthesized according to the type of assessment and reported attribution to the intervention, with formal safety evaluations distinguished from studies in which safety findings were reported incidentally. Therapeutic effects were synthesized by indication and outcome type, including favorable, neutral, and unfavorable findings. Efficacy comparisons were also examined to determine whether effects associated with lyophilization could be distinguished from concurrent differences in formulation, carrier, excipients, concentration, or delivery system.

Risk-of-bias findings were summarized descriptively at the domain level for all assessments and at the overall-assessment level where applicable for RoB 2 and ROBINS-I. Quantitative meta-analysis was not considered appropriate because the studies did not

provide sufficiently comparable interventions, comparators, models, and outcome measures. No pooled effect estimates were therefore calculated.

3. Results

3.1. Study Selection Results

The database searches identified 884 records. After removal of 491 duplicates, 393 unique records underwent title and abstract screening, of which 347 were excluded. Forty-six reports were sought for full-text retrieval. All were successfully retrieved and assessed for eligibility. Seven reports were excluded after full-text assessment: no eligible in vivo or human component ($n = 3$), no eligible disease, injury, tissue-repair, or regeneration model ($n = 2$), lyophilized MSC-derived product not evaluated as the in vivo therapeutic intervention ($n = 1$), and non-English full-text publication ($n = 1$). Full-text reports were excluded after eligibility assessment, and reasons for exclusion are listed in Supplementary Table S2. Thirty-nine studies met the eligibility criteria and were included in the systematic review. The study-selection process is presented in the PRISMA 2020 flow diagram (Figure 1).

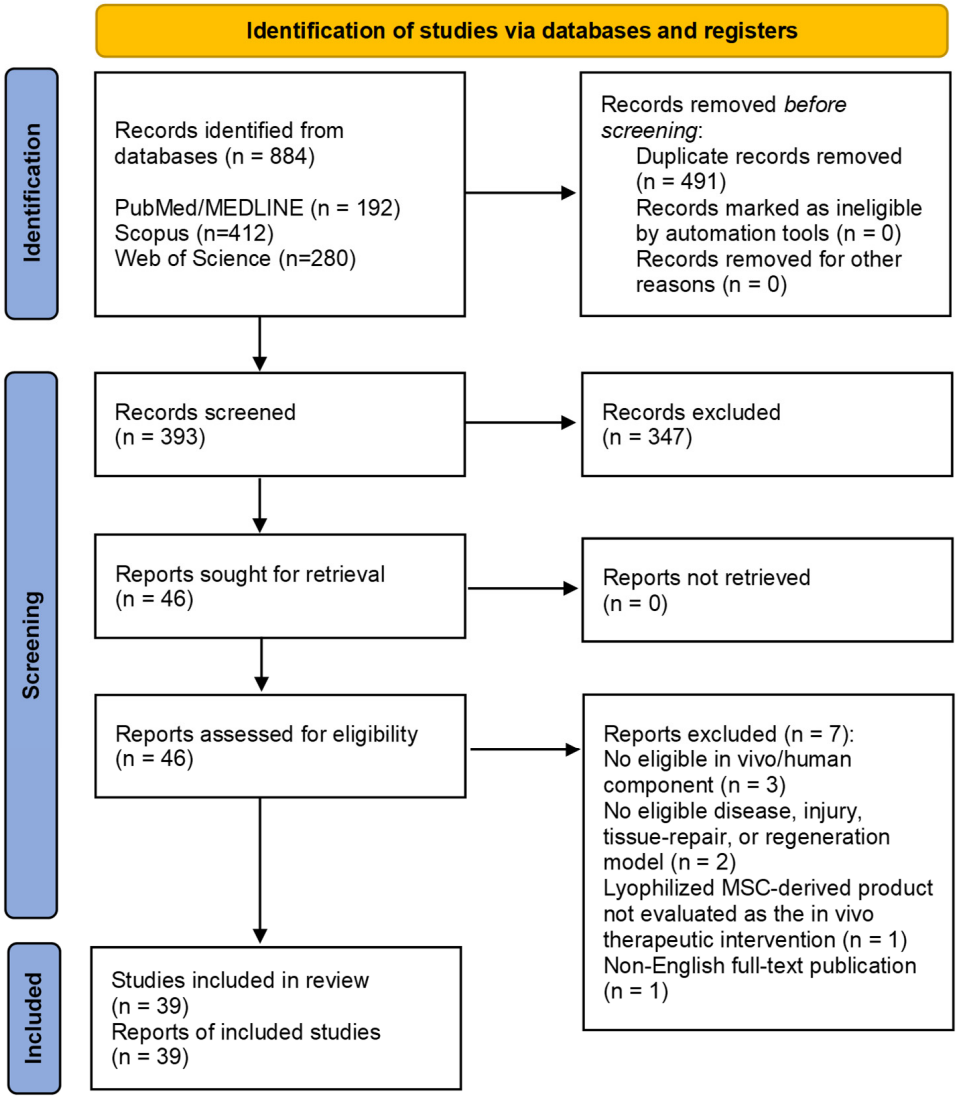


Figure 1. PRISMA flow diagram of study selection (adapted from the PRISMA 2020 flow diagram template).

3.2. Characteristics of Included Studies

The 39 included studies were predominantly preclinical. Thirty-four studies used experimental animal models, three investigated naturally occurring veterinary disease, one used a veterinary experimental model, and one combined preclinical experiments with a human clinical component (Table 1). Among experimental animal studies, cutaneous wound and soft-tissue models were the largest group (15/34), followed by inflammatory and organ-injury models (8/34), bone and periodontal regeneration (5/34), ocular and corneal regeneration (3/34), and other regenerative applications (3/34). Sample sizes varied widely across studies. Several studies used multiple experimental cohorts, treatment groups, or within-animal designs, precluding calculation of a single unambiguous sample size.

MSCs were derived from multiple tissue sources, including umbilical cord or Wharton's jelly, bone marrow, adipose tissue, placenta, dental tissues, and other sources. Both human- and animal-derived MSCs were used. Conditioned medium and secretome-based products were evaluated in 23 studies, extracellular vesicle and vesicle-containing products in 12, and whole-cell or synthetic MSC-based products in four.

Delivery routes varied according to indication and included local, topical, systemic, intra-articular, intrauterine, ocular, rectal, and oral administration. Follow-up ranged from several hours to several months, with the human study reporting outcomes up to 6 months.

Table 1. Study characteristics.

Study/ Reference	Country	Indication/ Model	N	MSC Source and Product	Intervention/ Comparator	Follow-Up
A. Mixed preclinical and human clinical evidence (n = 1)						
Jiang et al., 2025 [15]	China	Rat and human tooth extraction (hemostasis, bone regeneration)	Rats: 5–8/group, humans: 43 enrolled, 39 completed	Human umbilical cord MSC-derived lyophilized apoVs	MSC-apoV gelatin sponge/gelatin sponge (contralateral control in humans)	Rats: up to 3 months, humans: 6 months
B. Naturally occurring veterinary disease (n = 3)						
Díaz et al., 2025 [16]	Chile	Clinical endometritis in dairy cows	Safety: 12 cows, efficacy: 24 cows	Bovine fetal adipose MSC secretome, lyophilized	Lyophilized secretome/saline placebo/cefapirin benzathine	21 days
Berni et al., 2025 [17]	Italy	Spontaneous osteoarthritis in dogs, safety in horses	26 dogs, 5 horses	Canine/equine adipose MSC lyosecretome	Lyosecretome + hyaluronic acid/ mannitol + hyaluronic acid	80 days
Mocchi et al., 2021 [18]	Italy	Bilateral knee/elbow osteoarthritis in dogs	3 dogs	Canine adipose MSC lyosecretome	Lyosecretome + hyaluronic acid/ contralateral mannitol + hyaluronic acid	80 days
C. Veterinary experimental model (n = 1)						
Freitas et al., 2023 [19]	Brazil	Experimental skin wounds in horses	8 horses	Equine adipose MSC-CM, freeze-dried	Freeze-dried ASC-CM ± PRP/ saline/PRP	24 days

Table 1. *Cont.*

Study/ Reference	Country	Indication/ Model	N	MSC Source and Product	Intervention/ Comparator	Follow-Up
D. Experimental animal models (n = 34)						
Cutaneous wounds and soft-tissue regeneration						
Mahesh et al., 2026 [20]	India	Full-thickness skin wounds in Wistar rats	25 rats (5/group)	Bovine umbilical cord MSC-CM, lyophilized	Lyophilized CM/untreated/ vehicle/umbilical cord MSCs/ mupirocin	21 days
Chen et al., 2026 [21]	China	Full-thickness skin wounds in Sprague-Dawley rats	30 rats (10/group)	Human umbilical cord MSC-CM, lyophilized	Two lyophilized CM formulations/PBS	14 days
Zhang et al., 2024 (skin patch) [22]	China	Full-thickness skin defects in Sprague-Dawley rats	20 rats (3 wounds/ rat)	Rat nasal mucosa ecto-MSC-CM, lyophilized fibrin/chitosan patch	CM patch/CM-free patch/untreated	28 days
Wang et al., 2023 [23]	China	STZ-diabetic skin wounds in Sprague-Dawley rats	NR (4 wounds/ rat)	Human fetal MSC secretome, lyophilized in PLGA	HFS-PLGA/ untreated/vehicle/ PDGF-BB	21 days
Peng et al., 2015 [24]	China	Acute full-thickness skin wounds in Sprague-Dawley rats	15 rats (5 groups)	Rat bone marrow MSC-CM, freeze-dried membrane	Freeze-dried CM membrane/fresh CM/serum-free medium/ stabilization buffer/untreated	14 days
Sun et al., 2014 [25]	China	Full-thickness skin defects in Sprague-Dawley rats	30 rats (10/group)	Rat adipose MSC-CM, hypoxic, freeze-dried	Concentrated/ non-concentrated hypoxic CM/ vehicle	21 days
Shi et al., 2024 [26]	China	STZ-diabetic skin wounds in Sprague-Dawley rats	Total N NR	Human umbilical cord MSC exosomes, lyophilized Ga/ CSSF sponge	Ga/CSSF-Exo/Ga/ CSSF/untreated	21 days
Yang et al., 2024 (burn) [27]	China	Pseudomonas-infected deep second-degree burns in C57BL/ 6J mice	Main cohort: 8/group (5 groups), additional cohorts	Human umbilical cord MSC exosomes, lyophilized HD-DP7 hydrogel	HD-DP7/Exo/ PBS/hydrogel/HD-DP7/hydrogel-Exo	Healing: 17 days, scars: 28 days
Bulut et al., 2025 [28]	Turkey	Skin wounds in young/geriatric Wistar rats	72 rats (9/group)	Human dental follicle MSCs, lyophilized ± lyophilized PRF	Lyophilized DF-MSCs ± PRF/ untreated/PRF only	15 days
Hendrawan et al., 2024 [29]	Indonesia	STZ-diabetic splinted skin wounds in rats	10 rats (2/group, 2 wounds/ rat)	Human umbilical cord MSC-CM, freeze-dried PLLA/collagen matrix	CM-FD matrix ± fibroblasts/NaCl matrix/gentamicin matrix/CM	14 days

Table 1. *Cont.*

Study/ Reference	Country	Indication/ Model	N	MSC Source and Product	Intervention/ Comparator	Follow-Up
Widowati et al., 2026 [30]	Indonesia	Third-degree burns in Sprague-Dawley rats	36 rats (6/group)	Human Wharton's jelly MSC secretome, freeze-dried gel	FDSG regimens/ burn control/ gel control/ Bioplacenton	24 days
Duan et al., 2024 [31]	China	Diabetic full-thickness skin wounds in BALB/c mice	18 mice (3/group)	MSC supernatant lyophilized powder (source NR)	MSC-SLP dressing variants/base dressing/gauze	15 days
Joseph et al., 2020 [32]	India	Full-thickness skin wounds in guinea pigs	24 guinea pigs (6/group)	Canine, caprine and guinea-pig bone marrow MSC-CM, lyophilized	Species-specific lyophilized CM/ laminin-DMEM	28 days
Zhang et al., 2021 [33]	China	Rabbit-ear hypertrophic scars	12 rabbits	Rabbit adipose MSC-CM, freeze-dried	MSC-CM ± hydrogel/DMEM vehicle/dose controls	16 weeks
Tutak & Kıvrak, 2022 [34]	Turkey	Autologous fat grafts in rats	27 rats (9/group)	Human umbilical cord MSC whole-cell lyophilizate	Fat graft + MSC-L/fat graft + saline/native adipose tissue (no graft)	3 months
Bone and periodontal regeneration						
Kanazawa et al., 2026 [35]	Japan	Safety and ligature-induced periodontitis in mice	Safety: 7 × 6, efficacy: 8 × 3	Human dental pulp stem cell EV-containing CM, freeze-dried	Lyophilized CM/ vehicle/untreated	Safety: 14 days, efficacy: 3–7 days post-treatment
Buss et al., 2023 [36]	Brazil	Critical-size calvarial defects in rats	24 rats	Human dental pulp MSC-CM, lyophilized	Xenograft + lyophilized CM/ xenograft alone	42 days
Shanbhag et al., 2023 [37]	Austria	Critical-size calvarial defects in rats	20 enrolled, 17 entered experiment	Human bone marrow MSC-CM on collagen membrane	CM-LYO/CM-SOAK/native membrane/ MSC-seeded membrane/untreated	4 weeks
Huang et al., 2024 [38]	USA	Calvarial defects in rats	4 rats/group	Human bone marrow MSC engineered osteoinductive EVs, lyophilized	Lyophilized EVs/fresh EVs/rhBMP2/PBS	8 weeks
Liu et al., 2021 [39]	China	Critical-size cranial defects in rats	8 rats (2 groups)	Rat bone marrow MSC osteoinductive exosomes, lyophilized on MBG	BMSC-OI-exo-loaded MBG/ MBG alone	12 weeks

Table 1. *Cont.*

Study/ Reference	Country	Indication/ Model	N	MSC Source and Product	Intervention/ Comparator	Follow-Up
Ocular and corneal regeneration						
Jabbehdari et al., 2020 [40]	USA	Corneal epithelial wounds in mice	30 mice (10/group)	Human corneal MSC-CM, lyophilized	Lyophilized cMSC-CM/ fresh cMSC-CM/unconditioned medium	24 h
Fernandes-Cunha et al., 2019 [41]	USA	Mechanical corneal wounds and alkali burns in rats	Mechanical: 20, alkali burn: 28	Human bone marrow MSC secretome, lyophilized	MSC-S + hyaluronic acid/CS/MSC-S/ HA/CS/saline	Up to 14 days
Bahr et al., 2023 [42]	Egypt	Central and peripheral corneal ulcers in rabbits	24 rabbits (6/group)	Canine MSC-EVs, lyophilized	Lyophilized MSC-EVs/saline (both models)	3 weeks
Inflammatory and organ-injury models						
Yang et al., 2026 [43]	China	DSS-induced colitis in Sprague-Dawley rats	18 rats (6/group)	Human umbilical cord MSC-EVs, lyophilized	Lyophilized MSC-EVs/healthy control/DSS + PBS	11 days, biodistribution: 6–24 h
Oh et al., 2024 [44]	South Korea	*D. farinae*-induced atopic dermatitis in mice	Total N NR, multiple cohorts	Canine adipose MSC secretome/ CM, lyophilized	Secretome microspheres/ vehicle/blank microspheres/free secretome	30 days
Kudinov et al., 2022 [45]	Russia	LPS-induced lethal acute lung injury in mice	60 mice (10/group)	Human placental MSC secretome (2D/3D), freeze-dried	2D/3D MSC secretome/corresponding fibroblast secretome/saline	8 days
Peng et al., 2021 [46]	China	Bleomycin-induced acute lung injury in mice	6–8/group, total N NR	Human placental MSC-SLP	MSC-SLP/PBS/ bleomycin controls	7 days, mechanistic cohort: 2 days
Suzuki et al., 2026 [47]	Japan	D-galactosamine/ TNF-α-induced acute liver failure in mice	5/group, total N NR	Mouse bone marrow MSC-EVs, lyophilized	Lyophilized EVs/fresh EVs/ empty EVs/PBS/ non-ALF control	6 h
Yang et al., 2025 [48]	China	Radiation-induced enteritis in mice	Total N NR, multiple cohorts	Human umbilical cord MSC apoVs, lyophilized	Lyophilized apoVs/fresh apoVs/irradiated control	3 days, mechanistic: 1–24 h
Ebrahim et al., 2025 [49]	Egypt	SARS-CoV-2 infection in Syrian hamsters	28 hamsters (14/7/7)	Human Wharton's jelly MSC-EVs, lyophilized	Lyophilized MSC-EVs/untreated COVID-19/ uninfected controls	7 days
Kakabadze et al., 2022 [50]	Georgia	LPS-induced acute respiratory distress syndrome in rats	100 rats (20/group)	Human placental whole MSCs, freeze-dried	Lyophilized HPMSCs/LPS/dexamethasone/HPMSC-only/saline	12–168 h

Table 1. *Cont.*

Study/ Reference	Country	Indication/ Model	N	MSC Source and Product	Intervention/ Comparator	Follow-Up
Other regenerative applications						
Zhang et al., 2024 (ovarian failure) [51]	China	Cyclophosphamide-induced ovarian failure in rats	5–7/group (6 groups)	Human umbilical cord MSC 2D/3D EVs, lyophilized	2D- or 3D-MSC-EVs-ECM gel/CTX/ ECM/untreated	30 days
Jiang et al., 2023 [52]	China	Traumatic liver/ femoral-vessel bleeding in rats/rabbits	4/group, total N NR	Human umbilical cord MSC apoVs, lyophilized adhesive hydrogel sponge	apoV-HS/blank sponge/commercial hemostats/untreated	Acute hemostasis, biocompatibility up to 4 weeks
Luo et al., 2017 [53]	Japan	Acute myocardial infarction in C57BL/6 mice	8/group	Human bone marrow MSC synthetic particles (lyophilized CM-loaded PLGA core, MSC membrane coating)	Synthetic MSCs/ authentic MSCs/ untreated MI	15 days

Abbreviations: MSC—mesenchymal stromal cell, CM—conditioned medium, EV—extracellular vesicle, NR—not reported or not available as a single unambiguous total.

3.3. Characteristics of Lyophilized MSC-Derived Products

Preparation, formulation, and storage characteristics varied across the lyophilized MSC-derived products. In 22 of the 39 studies, MSC-derived products were incorporated into biomaterial delivery systems, including hydrogels, scaffolds, membranes, wound dressings, and microspheres (Supplementary Table S3).

Preparation methods for conditioned medium and secretome included centrifugation, filtration, ultrafiltration, tangential-flow filtration, and dialysis. Vesicle-based products were prepared using isolation or concentration procedures such as ultracentrifugation and tangential-flow filtration. In eight studies, the MSC-derived product was incorporated into a biomaterial matrix before the final freeze-drying step (Supplementary Table S3).

The level of reporting of lyophilization procedures was highly variable. Pre-freezing temperature was specified in nine studies, drying duration in 18, numerical chamber pressure in nine, and freeze-drying equipment in 14 of the 39 studies (Supplementary Table S3). Reported drying durations ranged from several hours to several days. Reported protocols included approximately 8 h of lyophilization after pre-freezing at −30 °C [47], 24 h of drying after pre-freezing at −80 °C [21], and 72-h cycles at −50 °C under reduced pressure [17,18].

Among the 12 studies evaluating EV- or vesicle-based therapeutic preparations, particle size distribution was assessed in 10 studies, particle concentration or counts in nine, vesicle morphology in 10, and EV-associated marker expression in nine. Aggregation or agglomeration was specifically examined in only three studies. Functional assessments included cellular uptake, migration, osteogenic and procoagulant activity, and other biological responses, although not all studies directly compared functional activity between lyophilized and non-lyophilized preparations. Characterization was particularly limited in studies using commercially supplied EV products or preparations with incompletely reported manufacturing procedures. Reported isolation methods included differential or ultracentrifugation, tangential-flow filtration, and polymer-based precipitation. Differences in isolation and characterization methods, together with inconsistent reporting, limited comparability between studies.

The use of lyoprotectants or stabilizing excipients was reported in 13 of the 39 studies, with their composition specified in 12 studies (Supplementary Table S3). Reported lyoprotectants included trehalose, used alone or with excipients such as PVP40 or mannitol [21,43]. Other reported protectants or stabilizing excipients included sucrose [47], DMSO [38], maltose monohydrate [45], and multicomponent stabilization buffers [24]. Dedicated lyoprotectants were not reported for the composite scaffold and hydrogel formulations described by Yang et al. [27] and Liu et al. [39].

Chen et al. [21] compared trehalose concentrations of 50–200 mM and formulations combining trehalose with PVP40 in lyophilized MSC-conditioned medium. The results indicated formulation-dependent preservation of particle characteristics and individual protein components. However, apparent increases in particle concentration were partly attributable to protein aggregates detected by nanoparticle tracking analysis. Suzuki et al. [47] compared MSC-derived extracellular vesicles lyophilized in PBS or with 1% or 5% sucrose. The 5% sucrose formulation better preserved vesicle morphology and particle-size distribution and showed a strong correlation with the miRNA profile of non-lyophilized EVs ($r = 0.95$). Huang et al. [38] investigated DMSO concentrations of 0%, 5%, and 10% in engineered MSC-derived EVs. Cellular uptake was substantially reduced after lyophilization without DMSO, partially restored with 5% DMSO, and approached that of fresh EVs with 10% DMSO. The remaining formulations included single protective formulations and biomaterial-based delivery systems, for which the individual contribution of lyoprotectants could not be established without direct comparative assessments.

The final lyophilized products ranged from dry powders intended for subsequent reconstitution to ready-to-use or rapidly rehydrating formulations incorporated into biomaterial carriers. Reconstitution or rehydration was described in 14 studies, using media such as sterile water, phosphate-buffered saline, physiological saline, or culture medium. Scaffold- and dressing-based formulations also included products intended for direct application. Dry-product storage temperature was specified in 15 studies and storage duration in 10 studies. Reported storage temperatures ranged from room temperature to −80 °C (Supplementary Table S3). Incomplete reporting of manufacturing parameters limited direct comparison of lyophilization strategies across studies.

3.4. Biological Activity and Stability After Lyophilization

Biological activity or stability after lyophilization was evaluated in 16 studies. Twelve studies directly compared lyophilized products with corresponding fresh or non-lyophilized preparations, providing direct evidence of retention through lyophilization [15,19,21,24,27,38–40,43,47,52,53] (Table 2). Four additional studies evaluated biological activity after lyophilization without an appropriate non-lyophilized comparator [18,28,32,37] (Table 3).

Direct-comparison studies demonstrated that structural, molecular, and functional properties could be preserved after lyophilization across conditioned medium, vesicle-based products, and engineered MSC-derived preparations. However, retention was formulation-dependent and was not uniform across biological endpoints. Protective excipients and biomaterial carriers contributed to the preservation of biological activity [21,27,38,39,47]. Reduced osteoinductive activity and structural alterations were observed in certain vesicle formulations, highlighting the limitations of unprotected lyophilization [38,39].

Storage-stability findings were available within the direct-comparison studies (Table 2). Although biological activity could be maintained during prolonged storage, the extent of preservation varied according to product formulation, storage temperature, and duration. Declines in biological activity or molecular-component concentrations were observed under

certain storage conditions [15,19,52]. Differences in stability endpoints and assessment intervals limited direct comparisons between products.

Studies without a non-lyophilized comparator demonstrated post-lyophilization biological activity, including enzymatic activity, regenerative effects, and cellular viability [18,28,32,37]. However, these findings did not establish the extent to which biological activity was retained through the lyophilization process.

Table 2. Direct evidence of biological activity retention and stability after lyophilization.

Study	Product	Comparison/Conditions	Key Findings	Storage Stability
Jiang et al., 2025 [15]	hUC-MSC-derived apoptotic vesicles (MSC-apoVs)	Fresh vs. freeze-dried MSC-apoVs, lyophilized preparations evaluated after storage at different temperatures	Freeze-dried apoVs retained procoagulant and osteogenic/regenerative functions. Preservation was maintained after storage at 4 °C or −80 °C. Procoagulant activity was lower after storage at 25 °C.	Functional properties retained for 3 months at 4 °C or −80 °C, poorer preservation at 25 °C.
Freitas et al., 2023 [19]	Equine adipose MSC-conditioned medium	Fresh vs. freeze-dried ASC-CM, freeze-dried preparations assessed after 90 and 180 days	Growth-factor concentrations were not significantly altered immediately after freeze-drying and remained similar through 90 days. A significant reduction was observed by day 180.	Growth-factor levels remained comparable through 90 days and decreased significantly by day 180.
Chen et al., 2026 [21]	hUC-MSC conditioned medium	Original CM vs. lyophilized CM without cryoprotectant (CM0), with trehalose (CM1), or with trehalose + PVP40 (CM2)	CM0 showed lower preservation of intact EVs, CM1/CM2 better preserved nanoparticle integrity and EV markers. Most soluble bioactive factors were comparable to Original CM. Changes were observed for individual cytokines.	Optimized CM2 retained core cytokine-related bioactivity after 1 year at −20 °C.
Peng et al., 2015 [24]	Fresh BMSC-CM vs. rehydrated freeze-dried BMSC-CM membrane (FBMSC-CMM)	Fresh BMSC-CM vs. stabilized freeze-dried CM membrane, additional SFM, stabilization-buffer membrane and untreated controls	FBMSC-CMM retained >80% of the measured paracrine factors relative to fresh BMSC-CM.	Not formally assessed.
Yang et al., 2024 [27]	HucMSC-Exo-loaded HD-DP7 hydrogel	Fresh exosomes vs. directly lyophilized exosomes vs. HD-DP7-protected/lyophilized exosomes, storage at RT or 4 °C for up to 120 days	At day 120, migration was 61.19 ± 2.62% after direct lyophilization vs. 74.44 ± 1.76% with HD-DP7 protection at RT. Differences at 4 °C were not significant. Antibacterial activity remained stable through 120 days.	Functional activity was maintained through 120 days. HD-DP7 improved preservation at RT.
Huang et al., 2024 [38]	Engineered osteoinductive MSC-EVs	Fresh engineered EVs vs. lyophilized EVs with DMSO cryoprotection, DMSO concentrations 0–10% evaluated	Optimized DMSO protection preserved EV size, markers, osteoinductive miRNAs, endocytosis and matrix binding. Most osteogenic/BMP2-pathway responses and in vivo bone regeneration were comparable to fresh EVs, although ALP activity decreased after lyophilization.	Not formally assessed.

Table 2. *Cont.*

Study	Product	Comparison/Conditions	Key Findings	Storage Stability
Liu et al., 2021 [39]	BMSC-derived osteoinductive exosomes on MBG scaffold	BMSC-OI-exosomes before lyophilization vs. directly lyophilized exosomes vs. MBG-scaffold-protected lyophilized exosomes	Direct lyophilization caused aggregation/disruption and significantly reduced ALP-based osteoinductivity. MBG-supported lyophilization preserved morphology, with only a slight, non-significant ALP reduction. Exosome release reached 71.40% at day 14 and 75.42% at day 28.	Not formally assessed.
Jabbehdari et al., 2020 [40]	Corneal MSC-conditioned medium	Fresh vs. lyophilized cMSC-CM in vitro and in a murine corneal epithelial injury model	Scratch closure was 82.2 ± 6.1% (fresh) vs. 86.3 ± 7.2% (lyophilized). Anti-inflammatory activity was comparable. In vivo wound healing was 87.6 ± 2.7% vs. 86.2 ± 4.6%, respectively.	Not formally assessed.
Yang et al., 2026 [43]	hUC-MSC extracellular vesicles	Non-lyophilized MSC-EVs vs. lyophilized MSC-EVs protected with 6% trehalose + mannitol, lyophilized EVs stored at 4 °C for 0, 3, 6, 9 and 12 months	Morphology, particle size and CD9/CD81/ALIX were preserved. CD9-positive EVs were 58.6% before vs. 56.3% after lyophilization. HUVEC scratch closure was 75.3 ± 2.0% vs. 76.1 ± 5.6%, respectively (NS), with comparable immunomodulatory effects.	Particle concentration and size showed no significant change through 12 months at 4 °C. Functional assays were not repeated at every storage time point.
Suzuki et al., 2026 [47]	Mouse BMSC-derived extracellular vesicles	Non-lyophilized EVs vs. EVs lyophilized in PBS, 1% sucrose or 5% sucrose	The 5% sucrose formulation showed the greatest preservation of EV number, morphology, RNA/protein content and miRNA profile. Correlation with non-lyophilized EV miRNA expression was 0.95, with 80 miRNAs showing stable expression. Therapeutic activity in acute liver failure was comparable to non-lyophilized EVs.	Not formally assessed.
Jiang et al., 2023 [52]	hUC-MSC apoptotic vesicles	Fresh apoVs vs. lyophilized apoVs, storage at −80 °C for 2 months or RT for 1–2 months	Diameter and TF/PS exposure were largely preserved, although particle number/membrane potential changed and TF slightly decreased. Fresh, −80 °C/2-month and RT/1-month preparations had similar procoagulant activity. Activity declined modestly after 2 months at RT.	Procoagulant activity was comparable to fresh apoVs after −80 °C for 2 months or RT for 1 month. A modest decline was observed after 2 months at RT.
Luo et al., 2017 [53]	Synthetic MSC particles (MSC-secretome-loaded PLGA core with MSC membrane coating)	synMSC before vs. after lyophilization	Lyophilization did not alter synMSC structure, size, surface-antigen expression, or sustained VEGF release.	Not formally assessed.

Table 3. Biological activity of lyophilized MSC-derived products without a non-lyophilized comparator.

Study	Product	Comparison/Conditions	Key Findings	Storage Stability
Mocchi et al., 2021 [18]	Canine adipose MSC secretome (Lyosecretome)	Reconstituted freeze-dried secretome evaluated across concentrations	Lyosecretome contained proteins, lipids and EV-sized particles and induced dose-dependent biological responses in canine MSCs, tenocytes and chondrocytes. Anti-elastase activity reached approximately 85% at 20 mg/mL.	Stored at −20 °C for up to 6 months. Longitudinal stability was not formally assessed.
Bulut et al., 2025 [28]	Lyophilized human dental-follicle MSCs ± PRF	Rehydrated lyophilized DF-MSCs alone vs. lyophilized DF-MSCs combined with PRF	Post-rehydration viability was 89.1 ± 7.3% for lyophilized DF-MSCs alone and 88.3 ± 4.9% with PRF. Cells retained differentiation/regenerative activity and promoted wound healing.	Not formally assessed.
Joseph et al., 2020 [32]	Lyophilized BM-MSC-conditioned media	Lyophilized canine, caprine and guinea-pig BM-MSC-CM preparations evaluated in a wound-healing model	Lyophilized MSC-CM preparations retained biological activity and promoted wound healing, but quantitative retention attributable specifically to lyophilization could not be determined.	Not formally assessed.
Shanbhag et al., 2023 [37]	hBMSC-CM-functionalized collagen membrane	CM-LYO (CM-soaked membrane followed by lyophilization) vs. CM-SOAK, native membrane, MSC-seeded membrane and untreated control	CM-LYO enhanced early bone regeneration. Proteomics of lyophilized CM identified 2684 proteins, including 255 associated with bone-related processes.	Not formally assessed.

3.5. Safety

Safety-related evidence was identified in 12 studies, including eight preclinical studies, three studies in veterinary patients, and one study with a human safety assessment (Table 4). Safety evaluations included dedicated toxicity and biocompatibility assessments, laboratory and histopathological examinations, and adverse-event monitoring. Studies in which adverse events were simply not reported were not considered to provide safety evidence.

Preclinical studies incorporating systemic toxicity or biocompatibility assessments identified no major systemic toxicity signals under the conditions evaluated [31,35,52]. Additional safety observations within therapeutic studies were generally favorable, although dedicated toxicological assessments were not performed [37,44].

Concentration-dependent adverse cellular effects were reported in three studies [21,33,41], including reduced cell viability, inhibition of fibroblast proliferation, and increased apoptosis. Delayed wound closure was also observed at high conditioned-medium concentrations [33]. Increased osmolality and alkaline pH were identified as potential contributing factors [21,41], limiting attribution of these effects specifically to MSC-derived biological components.

Veterinary studies reported generally favorable systemic tolerability [16–18]. Transient local reactions, including joint swelling, inflammation, and locomotor discomfort, were observed after intra-articular administration [17,18]. Similar reactions in control-treated joints and the absence of a consistent dose-dependent pattern limited attribution of these events specifically to the MSC-derived secretome.

Human safety evidence was limited to one study, in which no treatment-related serious adverse events were reported during the six-month follow-up [15].

Table 4. Safety findings of lyophilized MSC-derived products.

Study	Population/Model	Safety Assessment	Main Safety Findings
A. Preclinical safety evidence			
Chen et al., 2026 [21]	L929 fibroblasts and HDFs, rat full-thickness skin-wound model	In vitro cell-viability/cytotoxicity testing across Lyo-CM formulations and concentrations, no dedicated in vivo systemic safety assessment.	CM0 showed no cytotoxicity in L929 cells. CM1 and CM2 reduced cell viability to approximately 85% at the highest concentration (2×). CM2 at 2× had increased osmolality (340 mOsm/kg). Lower concentrations were not associated with this effect.
Kanazawa et al., 2026 [35]	Male and female mice, intravenous and gingival administration of freeze-dried DPSC-CM	Single-dose toxicity assessment with 2-week observation, general condition, body weight, hematology, blood biochemistry, and necropsy.	No adverse changes in general condition, body weight, or necropsy findings. Hematological and biochemical differences between groups were not considered toxicologically significant.
Oh et al., 2024 [44]	Mice with *D. farinae*-induced atopic dermatitis	Monitoring of respiratory abnormalities, survival, body weight, and major-organ abnormalities during the efficacy study No dedicated toxicity testing.	No significant respiratory, survival, body-weight, or major-organ abnormalities were observed. Body weight did not differ significantly between ST/ST-CS-MS and control groups.
Shanbhag et al., 2023 [37]	Lewis rats with bilateral critical-size calvarial defects	Postoperative recovery and adverse-event monitoring during the 4-week study. No dedicated toxicological assessment.	All animals entering the experimental phase recovered after surgery, with no adverse events observed. Three of 20 rats died from anesthesia-related complications before treatment allocation.
Zhang et al., 2021 [33]	Human keloid fibroblasts, New Zealand rabbit ear hypertrophic-scar model	Fibroblast proliferation and apoptosis across CM concentrations. In vivo wound-healing observations Systemic toxicity was not assessed.	CM10 inhibited fibroblast proliferation and increased apoptosis. CM10 and CM10 + hydrogel delayed wound closure versus blank control ($p < 0.05$), with a prolonged inflammatory/proliferative phase.
Fernandes-Cunha et al., 2019 [41]	Primary human corneal epithelial cells, rat mechanical and alkaline corneal injury models	In vitro Live/Dead cytotoxicity testing (24–72 h) and in vivo ocular observations. No dedicated systemic toxicity assessment.	No significant cell death at the selected concentration (1.25 mg/mL). The highest concentration (15 mg/mL) appeared cytotoxic, potentially related to alkaline pH (9.0).
Jiang et al., 2023 [52]	L929 fibroblasts, rats with dorsal subcutaneous implantation	In vitro cytocompatibility (CCK-8, EdU), hemolysis testing, and in vivo local biocompatibility with histology and CD3+ T-cell infiltration at 2 and 4 weeks. Systemic toxicity was not assessed.	apoV-HS did not impair L929 proliferation. Hemolysis was 0.51 ± 0.1%. Implantation elicited very mild local inflammation, with no significant difference in CD3+ T-cell infiltration versus control.
Duan et al., 2024 [31]	Mice with cutaneous wounds	In vitro/in vivo biocompatibility assessment, serum biochemistry and major-organ histology (heart, liver, spleen, lung, and kidney) at Day 15.	No significant between-group differences in serum biochemical indicators or treatment-associated pathological abnormalities in major organs at Day 15.

Table 4. *Cont.*

Study	Population/Model	Safety Assessment	Main Safety Findings
B. Veterinary safety evidence			
Díaz et al., 2025 [16]	Healthy first-calving Holstein cows ($n = 12$, sMSC $n = 6$, placebo $n = 6$)	Twenty-one-day prospective safety study after two intrauterine administrations, clinical parameters, endometrial cytology, hematology, and serum biochemistry.	No clinical adverse reactions were observed, and clinical variables remained within physiological ranges. Transient hematological and biochemical changes were predominantly within reference ranges. Endometrial cytology showed no local inflammatory response.
Berni et al., 2025 [17]	Dogs ($n = 26$) and horses ($n = 5$) with naturally occurring osteoarthritis	Clinical and orthopedic monitoring after intra-articular lyosecretome; preliminary equine safety assessment included local/general examination and synovial-fluid analysis; follow-up up to 80 days.	No systemic adverse reactions. Transient mild-to-moderate joint swelling/inflammation was self-limiting, also occurred with control treatment, and was not dose-dependent. Synovial-fluid analysis showed no significant treatment-related changes.
Mocchi et al., 2021 [18]	Dogs with bilateral naturally occurring osteoarthritis (5 enrolled in safety study)	Within-animal controlled safety evaluation after intra-articular. Lyosecretome versus placebo, clinical monitoring and questionnaire-based assessment of undesired effects after repeated administration.	No systemic adverse reactions, including after the second administration. Transient reluctance to trot or gallop resolved within 2 days without treatment. One dog developed bilateral swelling and discomfort in both lyosecretome- and placebo-treated joints.
C. Human safety evidence			
Jiang et al., 2025 [15]	Patients undergoing bilateral impacted mandibular third-molar extraction (43 enrolled, 39 completed follow-up)	Double-blind, randomized, self-controlled, single-dose pilot trial, allergy testing, clinical monitoring, and hematological, liver- and kidney-function testing through 6 months.	All 39 completers tolerated MSC-apoVs well. No serious adverse events, alveolar osteitis, or other serious abnormalities were observed. Six-month hematological, liver- and kidney-function results were normal, clinically insignificant, or consistent with pretreatment values.

Overall, the available evidence did not identify a major systemic toxicity signal but remained predominantly preclinical and heterogeneous in scope. The limited number of studies, differences in safety assessments, and scarcity of human data precluded reliable conclusions regarding rare, delayed, or product-specific adverse effects.

3.6. Therapeutic Effects

Therapeutic effects were evaluated in cutaneous and soft-tissue repair, bone and periodontal regeneration, ocular and corneal injury, inflammatory and organ-injury models, and several other regenerative applications. Findings were synthesized by therapeutic application, with numerical outcomes reported in Supplementary Table S4.

3.6.1. Cutaneous Wound and Soft-Tissue Regeneration

Cutaneous wound and soft-tissue regeneration was the largest efficacy category. Most studies reported improvement in at least one measure of wound repair, including wound closure, re-epithelialization, angiogenesis, collagen organization, or tissue remodeling. Beneficial effects were reported for reconstituted conditioned media and secretomes as well as products incorporated into membranes, hydrogels, scaffolds, microspheres, and composite wound dressings (Supplementary Table S4).

Several studies reported substantial differences in wound closure. Chen et al. [21] found approximately 75.0% wound-area reduction by Day 7 with an optimized lyophilized hUC-MSC conditioned-medium formulation compared with 41.6% in controls. Peng et al. [24] reported faster wound closure with a freeze-dried BMSC-conditioned-medium membrane than with untreated wounds or fresh conditioned medium, while Duan et al. [31] reported more than 30% greater healing with a dressing containing MSC-supernatant lyophilized powder than with medical gauze. Exosome-containing scaffolds and hydrogels also improved wound closure and tissue-repair outcomes in several models.

The findings were not uniformly favorable. Freitas et al. [19] found no significant improvement in healing time or histopathological outcomes in experimentally induced equine cutaneous wounds treated with freeze-dried adipose MSC-conditioned medium, although a non-significant trend toward faster healing was observed in the PRP/ASC-CM groups. In a fat-graft model, Tutak and Kıvrak [34] reported reduced graft weight and volume loss and better tissue preservation with MSC lyophilizate compared with the control treatment.

3.6.2. Bone and Periodontal Regeneration

Bone and periodontal studies generally reported increased bone formation or related regenerative responses, although the magnitude and consistency of effects varied between models. Shanbhag et al. [37] reported 85.5 ± 15.49% bone coverage at 2 weeks with a collagen membrane functionalized with concentrated MSC-conditioned medium and subsequently lyophilized, compared with 21.67 ± 21.13% with a soaked conditioned-medium membrane ($p < 0.001$), with greater regeneration persisting at 4 weeks. Because the interventions also differed in product immobilization and delivery, this difference cannot be attributed to lyophilization alone. Buss et al. [36] also reported new bone formation and increased early vascularization with lyophilized dental-pulp MSC-conditioned medium combined with an osteoconductive scaffold.

EV-based formulations showed similar regenerative potential. Liu et al. [39] found increased bone volume fraction and trabecular thickness with exosomes immobilized on a mesoporous bioactive-glass scaffold compared with scaffold alone. Huang et al. [38] found no significant difference in in vivo bone regeneration between fresh and optimized lyophilized engineered BMP2 MSC-EVs, supporting retention of therapeutic activity under the tested conditions. Periodontal findings were less consistent: Kanazawa et al. [35] reported reduced inflammation and osteoclast activity and improved alveolar bone-surface morphology with freeze-dried dental-pulp MSC-conditioned medium, but clear recovery of crestal bone height was not demonstrated.

Human efficacy evidence in this category was limited to Jiang et al. [15]. In patients undergoing bilateral third-molar extraction, a lyophilized UC-MSC apoptotic-vesicle gelatin sponge shortened hemostatic time and accelerated alveolar regeneration, including greater trabecular thickness, whereas postoperative pain and swelling were comparable between treated and control sites.

3.6.3. Ocular and Corneal Regeneration

Three studies evaluated ocular or corneal regeneration, and each reported improvement in at least one regenerative outcome. Jabbehdari et al. [40] found comparable activity of fresh and lyophilized human corneal MSC-conditioned medium, with in vitro wound closure of 82.2 ± 6.1% and 86.3 ± 7.2%, respectively, and similarly comparable in vivo closure. Fernandes-Cunha et al. [41] reported accelerated epithelial closure and reductions in scarring, neovascularization, and hemorrhage with lyophilized BM-MSC secretome incorporated into a hyaluronic acid/chondroitin-sulfate gel in mechanical and alkali-burn

corneal injury models. Bahr et al. [42] found faster healing of central and peripheral corneal ulcers after topical administration of lyophilized canine MSC-EVs: treated corneas became fluorescein-negative by Day 5 and achieved complete epithelialization within one week.

3.6.4. Inflammatory and Organ-Injury Models

In gastrointestinal models, lyophilized MSC-derived products improved several structural, functional, and inflammatory outcomes. Yang et al. [43] reported reduced disease activity and histological injury, restoration of intestinal barrier markers, and modulation of inflammatory mediators after oral administration of lyophilized hUC-MSC EVs in DSS-induced colitis. Yang et al. [48] similarly reported improvement in structural and functional indices of radiation enteritis with lyophilized apoptotic vesicles, accompanied by reductions in oxidative stress and DNA damage.

Pulmonary models included acute lung injury, ARDS, and SARS-CoV-2-associated lung injury. Kudinov et al. [45] reported improved survival and reduced histological injury, edema/exudation, and fibrin deposition with freeze-dried placental MSC secretome in a lethal LPS model. Peng et al. [46] reported reduced inflammatory injury and enhanced epithelial repair with MSC-supernatant lyophilized powder in bleomycin-induced lung injury. Ebrahim et al. [49] found improved lung histoarchitecture and inflammatory and coagulopathy markers with lyophilized WJ-MSC EVs in SARS-CoV-2-infected hamsters, while Kakabadze et al. [50] reported attenuation of inflammatory, edematous, and fibrotic changes with freeze-dried placental MSCs in experimental ARDS.

In acute liver failure, Suzuki et al. [47] found that 5% sucrose-protected lyophilized BMSC-EVs reduced biochemical and inflammatory indices and hepatocyte injury. Therapeutic effects were comparable to those of non-lyophilized EVs, providing direct evidence of preserved efficacy under the tested lyophilization conditions.

3.6.5. Other Regenerative Applications

Additional applications included bovine endometritis, spontaneous osteoarthritis, chemotherapy-induced ovarian failure, myocardial infarction, atopic dermatitis, and traumatic hemorrhage. Oh et al. [44] also reported improvement in clinical and inflammatory outcomes in an experimental atopic-dermatitis model. In bovine endometritis, Díaz et al. [16] reported reductions of approximately 47% in endometrial polymorphonuclear cells and 78% in endometritis grade after intrauterine administration of lyophilized MSC secretome compared with placebo, with effects broadly comparable to cefapirin.

Findings in spontaneous osteoarthritis were less conclusive. Berni et al. [17] reported improvements in lameness and functional activity after lyosecretome administration, but significant superiority for pain reduction was not demonstrated. Mocchi et al. [18] primarily evaluated safety in a three-dog proof-of-principle study and did not establish therapeutic effects.

In chemotherapy-induced ovarian failure, Zhang et al. [51] reported improvement in ovarian-function markers with both 2D- and 3D-hUC-MSC-EV formulations, with greater effects for the 3D-derived formulation. Luo et al. [53] found that lyophilization-stable synthetic MSC particles reduced infarct size and promoted regenerative responses after myocardial infarction. Lyophilized apoptotic-vesicle hydrogel also showed hemostatic activity in a severe hemorrhage model: Jiang et al. [52] reported hemostasis within 2.5 ± 0.58 min and blood loss of 7.18 ± 2.56 g in treated rabbits, whereas secondary bleeding occurred in the Surgifoam comparator group.

Most studies reported improvement in at least one regenerative or functional outcome, but neutral or mixed findings occurred in cutaneous wound healing, periodontal regeneration, and veterinary osteoarthritis. Direct comparisons of therapeutic effects between

lyophilized and corresponding non-lyophilized products were uncommon. Where such comparisons were available, some formulations showed comparable efficacy after lyophilization, but the evidence was insufficient to generalize this finding across product types or therapeutic applications.

3.7. Overall Evidence Pattern

The evidence base was substantially broader for therapeutic effects than for the specific effects of lyophilization on MSC-derived products. Although regenerative or functional outcomes were evaluated in nearly all included studies, only 16 specifically examined direct retention through lyophilization, storage stability, or post-lyophilization biological potency. Direct comparisons with corresponding fresh or non-lyophilized products were available in 12 studies. These comparisons showed that preservation of structural, molecular, and functional properties was achievable, but was influenced by formulation, protective excipients, carriers, and storage conditions. Partial loss of specific properties was observed in some preparations, particularly when lyophilization was performed without adequate protection.

Evidence addressing safety and therapeutic effects within the same experimental framework was less consistent. Safety-related outcomes were evaluated in 12 studies and ranged from dedicated toxicity and biocompatibility assessments to adverse-event monitoring alongside efficacy experiments. No major systemic toxicity signal emerged from the available studies, but concentration-dependent cytotoxicity and transient local reactions were reported. Most efficacy studies reported improvement in at least one regenerative or functional outcome, although neutral or mixed findings occurred in several applications. Consequently, evidence of therapeutic activity after lyophilization was considerably more common than evidence demonstrating that efficacy had been preserved specifically through the lyophilization process.

The evidence therefore remains concentrated at the preclinical stage. Veterinary studies provided limited information from naturally occurring disease, and only one study included a human interventional component. Few studies evaluated product characteristics before and after lyophilization together with storage stability, safety, and therapeutic effects. The principal evidence gap is therefore not whether lyophilized MSC-derived products can remain biologically active, but whether reproducible preservation, defined storage stability, safety, and therapeutic effects can be demonstrated within the same product and subsequently confirmed in clinically relevant studies. An overview of the therapeutic evidence by major application, experimental and clinical models, main findings, and remaining evidence gaps is presented in Figure 2.

3.8. Risk of Bias

Risk of bias was assessed separately for each eligible study component using design-specific tools. Thirty-six animal-study components were assessed using the SYRCLE risk-of-bias tool, including the animal component of Jiang et al. [15]. Three randomized veterinary or human study components were assessed using RoB 2, and the non-randomized veterinary study by Mocchi et al. [18] was assessed using ROBINS-I. Because Jiang et al. [15] included eligible animal and human components with different study designs, the 39 included studies generated 40 design-specific assessments. The distribution of domain-level risk-of-bias judgements across the included study components is summarized in Figure 3. Detailed risk-of-bias assessments, including domain-level judgements and supporting explanations, are provided in Supplementary Table S5.

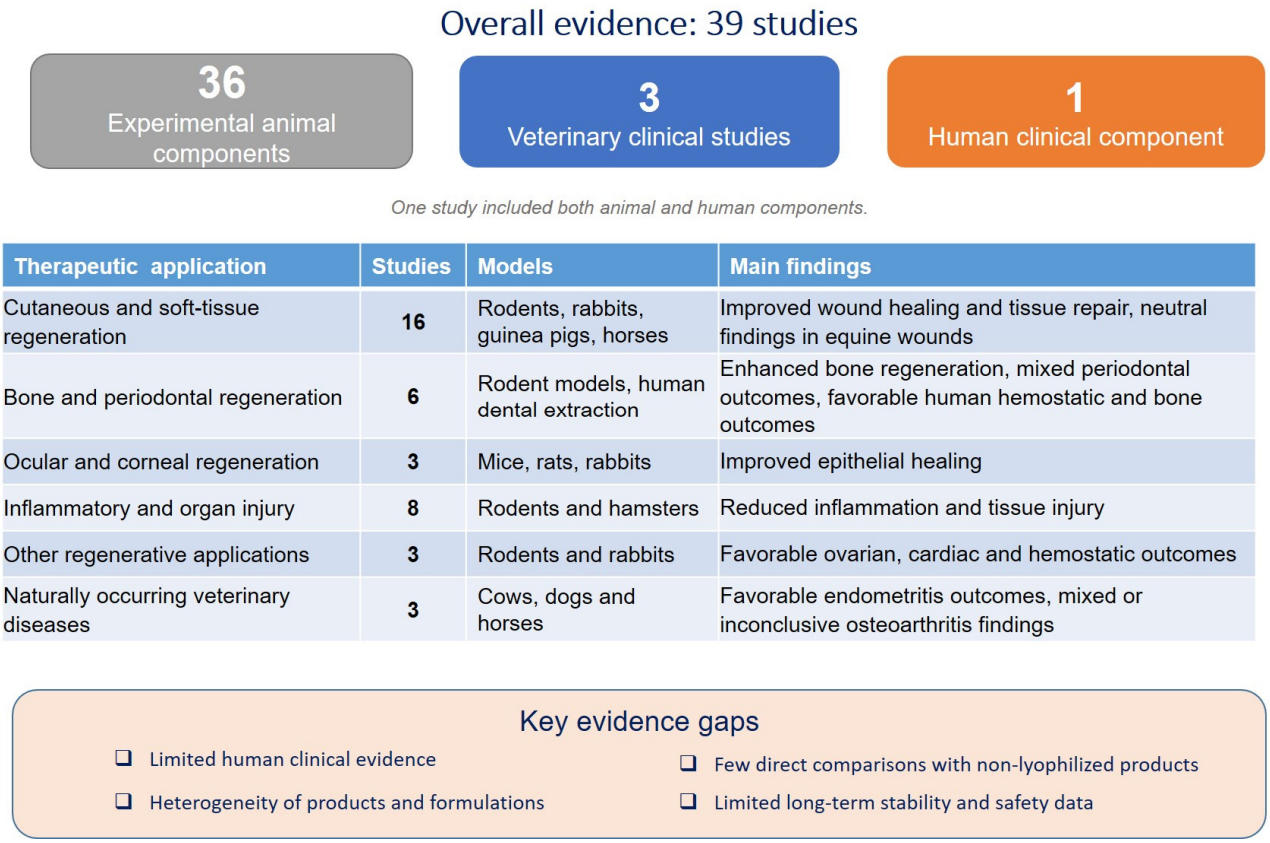


Figure 2. Overview of therapeutic evidence for lyophilized MSC-derived products across major therapeutic applications, experimental and clinical models, main findings, and key evidence gaps.

The predominant limitation among animal studies was insufficient reporting of measures intended to reduce selection and detection bias. Random sequence generation was judged at low risk in only 1 of 36 assessments and was unclear in the remaining 35. Baseline characteristics were judged to be at low risk of bias in five of the 36 animal-study components (13.9%) and at unclear risk in the remaining 31 (86.1%), primarily because group-specific baseline characteristics were insufficiently reported. Random outcome assessment was judged at low risk in four studies and blinded outcome assessment in six, with the remaining assessments predominantly unclear because the relevant procedures were not reported. In contrast, incomplete outcome data were judged at low risk in 30 of 36 assessments and other sources of bias in 34. Selective outcome reporting was judged to be at low risk of bias in 25 animal-study components (69.4%) and at unclear risk in 11 (30.6%). No SYRCLE domain was explicitly judged at high risk. The predominance of unclear judgements therefore reflected limited methodological reporting rather than documented evidence that these procedures had been performed inadequately.

All three randomized veterinary or human study components assessed with RoB 2 were judged to have some concerns rather than low or high overall risk of bias. The non-randomized veterinary study by Mocchi et al. [18] was judged at moderate overall risk of bias using ROBINS-I.

Taken together, the risk-of-bias assessment did not identify a consistent pattern of explicitly high-risk domains, but confidence in the evidence was limited by incomplete reporting of key methodological safeguards, particularly randomization and blinding in animal studies. The small number of randomized veterinary and human studies and the moderate risk of bias in the non-randomized veterinary study further limit the strength of conclusions drawn from the translational evidence. These limitations should be considered when interpreting the predominantly favorable efficacy findings.

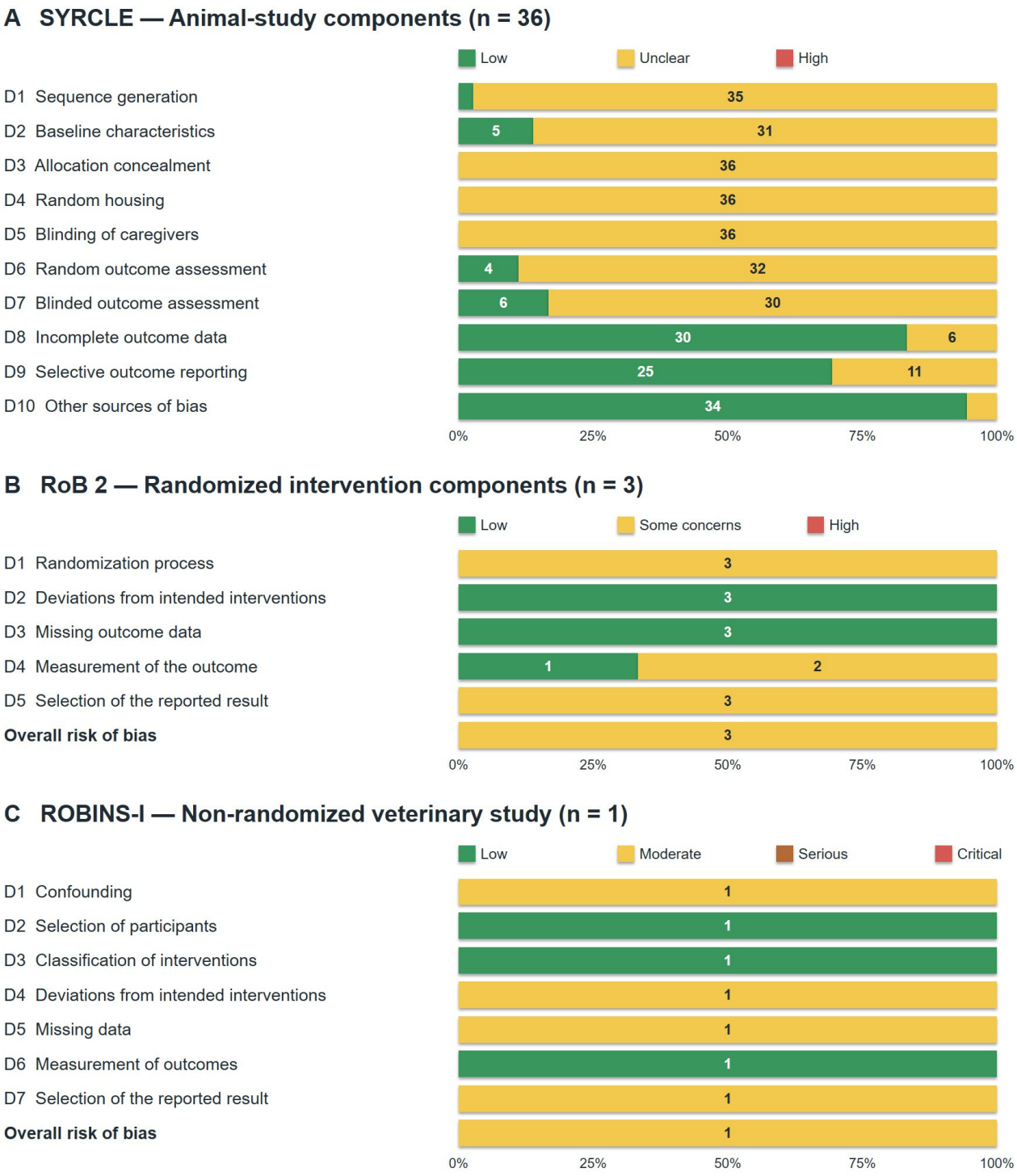


Figure 3. Domain-level risk-of-bias assessments of the included study components. (**A**) SYRCLE assessments of experimental animal-study components ($n = 36$), (**B**) RoB 2 assessments of randomized interventional components ($n = 3$), and (**C**) ROBINS-I assessment of the non-randomized veterinary intervention study ($n = 1$).

4. Discussion

This systematic review included 39 studies evaluating lyophilized MSC-derived products across a broad range of regenerative medicine applications. The available evidence indicates that structural, molecular, and biological properties can be preserved after lyophilization in at least some MSC-derived products, and that lyophilized preparations can remain therapeutically active in vivo. However, direct evidence that lyophilization preserves these

properties was considerably more limited than the overall number of included studies might suggest. The predominantly preclinical nature of the evidence further limits its clinical applicability. The current evidence supports the feasibility of lyophilization for the development of MSC-derived therapeutic products, but is insufficient to establish that biological activity, safety, and therapeutic performance are consistently maintained across product types, formulations, and applications.

Evidence of biological activity after lyophilization should be distinguished from evidence that biological activity was retained through the lyophilization process. Direct comparisons demonstrated that structural, molecular, and functional properties of MSC-derived EVs can be preserved under appropriate lyophilization conditions [43,47]. However, incomplete preservation of osteogenic activity and exosome aggregation were also reported [38,39]. These differences indicate that preservation of structural and molecular characteristics does not necessarily ensure retention of functional potency. These observations are consistent with previous reviews and current consensus recommendations emphasizing the influence of formulation, processing, and storage conditions on EV integrity and biological activity [4,54,55]. Against this background, post-lyophilization activity alone cannot be interpreted as evidence of retained activity unless an appropriate fresh or non-lyophilized comparator is available. Total protein concentration and particle count provide information on product recovery but are insufficient as measures of functional potency. Total protein measurements do not distinguish biologically active components from inactive or degraded proteins, while particle counts may include aggregates and do not establish vesicle integrity or biological activity. Therefore, assessment of potency requires product-specific functional assays relevant to the intended biological activity.

Differences in product formulation complicated attribution of both preservation and therapeutic effects to lyophilization. Protectants and other excipients, product concentration, and carrier or delivery systems frequently differed between experimental formulations and therefore formed part of the intervention rather than passive background variables. Comparisons of different lyoprotectant formulations [21] and scaffold-based delivery strategies [39] illustrate the importance of formulation design for preserving biological activity. Similarly, differences in bone regeneration between lyophilized and soaked MSC-conditioned-medium collagen membranes cannot be attributed exclusively to lyophilization because product immobilization and delivery also differed [37]. This interpretation is supported by the pharmaceutical lyophilization literature, which shows that preservation of complex biological products depends on the composition and concentration of stabilizing excipients and their interaction with freeze-drying process parameters [5,6]. Scaffold- and hydrogel-based delivery systems can also alter local retention, release kinetics, exposure duration, and bioavailability of MSC-derived secretomes independently of lyophilization [7]. Other emerging biomaterial-based platforms, such as layered double hydroxide-based composites, are also being explored for bone regeneration and wound healing [56]. The available evidence is therefore insufficient to identify an optimal protectant or formulation, while the lack of standardized formulations and lyophilization protocols limits cross-study comparison. Observed preservation or therapeutic effects should therefore be interpreted as an effect of the complete product-formulation-delivery system unless lyophilization is the only experimental variable that differs between comparison groups.

Lyophilization may facilitate the storage and distribution of MSC-derived therapeutics by enabling dry formulations with the potential for extended shelf life and reduced dependence on continuous frozen storage. However, a reported storage condition should not be equated with demonstrated stability. While functional preservation was shown to depend on storage temperature [15], long-term physicochemical stability did not necessarily establish sustained biological potency when functional assays were not repeated

throughout the storage period [43]. Although carrier-assisted lyophilization and optimized lyoprotectant formulations supported prolonged functional preservation [21,27], time-dependent deterioration in other preparations [19] indicates that stability remains product- and formulation-specific. These findings are consistent with the broader EV literature and current consensus recommendations emphasizing the need to experimentally assess the effects of storage conditions on EV characteristics and function [4,54]. In accordance with established stability principles for biological products [57], claims of extended shelf life or off-the-shelf availability require product-specific stability data collected over defined storage periods and incorporating functional and physicochemical endpoints. Until such evidence is available, reduced dependence on frozen storage should be regarded as a potential rather than an established advantage of lyophilization.

The available evidence did not identify a major systemic toxicity signal but was insufficient to establish the safety profile of lyophilized MSC-derived products. Dedicated or systematic safety monitoring was performed in only a subset of studies. Although findings from human, veterinary, and preclinical toxicological studies were generally reassuring [15–17,35], differences in product formulations, routes of administration, exposure duration, and safety monitoring limit their integration into a comprehensive safety assessment. Concentration-dependent cytotoxicity was identified in vitro in some formulations, indicating that tolerability may depend on dose, formulation, and physicochemical properties even when corresponding in vivo toxicity has not been demonstrated. In particular, increased osmolality and alkaline pH were identified as potential contributors to reduced cell viability, complicating the attribution of these effects specifically to MSC-derived biological components. The absence of reported adverse events cannot be interpreted as evidence of absence of toxicity when safety monitoring was not dedicated, systematic, or sufficiently reported. Clinical studies of extracellular vesicle therapies have similarly reported a low frequency of serious adverse events, although pooled analyses have noted substantial heterogeneity in study design, manufacturing methods, and adverse-event reporting [58]. Clinical translation therefore requires product-specific safety evaluation, including prospectively defined and systematically reported assessments of adverse events, laboratory toxicity, immunogenicity, dose-related effects, and longer-term complications.

Although favorable therapeutic effects were reported across diverse regenerative applications, the evidence was heterogeneous and predominantly preclinical. However, differences in product type and formulation, dose and administration, experimental models, comparators, and outcome assessment prevented meaningful comparison of effect magnitude across studies. Favorable therapeutic outcomes observed with scaffold- and hydrogel-based formulations [27,37] reflect the performance of the complete therapeutic system, whereas direct comparisons with non-lyophilized preparations provide more specific evidence of retained therapeutic activity [47]. The absence of significant therapeutic improvement in some experimental settings [19] further argues against assuming a uniform therapeutic benefit across products and applications. The only human interventional study provides an initial proof of concept for local clinical application [15], but its exploratory design and limited sample size preclude definitive conclusions regarding clinical effectiveness. Systematic reviews of MSC-conditioned medium and MSC-derived extracellular vesicles have also reported favorable regenerative effects across multiple preclinical models, while emphasizing substantial methodological heterogeneity and the need for standardized manufacturing, characterization, and outcome assessment [59,60]. Reviews of the MSC-EV clinical-trial landscape similarly identify variability in EV characterization, dosing metrics, routes of administration, and outcome measures as major barriers to clinical translation [61]. Clinical development should therefore prioritize specific product–indication combinations rather than extrapolating favorable findings across heterogeneous experimental models.

Clinical translation now requires demonstration that these effects are reproducible with standardized products, doses, and formulations and are maintained for clinically relevant endpoints in adequately powered human trials.

Interpretation of the favorable efficacy findings is limited by incomplete methodological reporting rather than by a consistent pattern of explicitly high-risk judgements. Allocation concealment, random housing, and blinding of caregivers or investigators were generally insufficiently reported. The absence of high-risk judgements should not be interpreted as evidence of low risk of bias, because insufficient reporting frequently prevented verification that key safeguards against bias had been implemented. This limitation is particularly relevant in light of ARRIVE 2.0, which identifies transparent reporting of randomization, blinding, sample-size determination, outcome measures, and inclusion and exclusion criteria as essential for evaluating the reliability and reproducibility of animal experiments [62]. Similar deficiencies have been documented in previous systematic reviews of preclinical MSC research, in which random-sequence generation, allocation concealment, random housing, and blinding were also infrequently reported [63]. These methodological safeguards also affect the validity of estimated treatment effects. Meta-research in animal studies has shown that inadequate randomization and the absence of other measures to reduce bias can be associated with exaggerated estimates of treatment efficacy [64]. Methodological concerns were also present in the limited veterinary and human evidence. Small experimental groups in many studies may further reduce the precision and reproducibility of effect estimates, although imprecision should be distinguished from risk of bias itself. These limitations reduce confidence in the overall preclinical efficacy evidence and emphasize the need for replication in transparently reported studies with adequate randomization, blinding, prespecified outcomes, appropriate sample-size justification, and rigorous handling of exclusions and missing data [12,62].

This review has several strengths. The review was prospectively registered in PROSPERO. The final search covered PubMed, Scopus, and Web of Science without publication-date restrictions. The broad scope enabled biological activity and stability after lyophilization, safety, and therapeutic effects to be evaluated within a single evidence framework across different MSC sources and product types. Operational definitions distinguished post-lyophilization potency from demonstrated retention and reported storage conditions from formal stability evidence, while safety findings were classified according to their context and reported attribution. Risk of bias was evaluated using design-specific tools appropriate to the experimental, veterinary, and human evidence. Nevertheless, several limitations should be considered. Substantial heterogeneity in MSC source, product type, lyophilization procedure, protectants and excipients, formulation and carrier, dose, route of administration, disease model, comparator, follow-up, and outcome assessment limited direct comparison between studies and precluded meaningful quantitative pooling. Even among studies addressing similar therapeutic indications, such as cutaneous wound healing and bone regeneration, differences in MSC-derived product formulations, experimental models, comparator interventions, and outcome assessment time points prevented meaningful pooling of the results. The evidence base was predominantly preclinical, with limited investigation in naturally occurring veterinary disease and only one study including a human interventional component, restricting clinical generalizability. Reporting of manufacturing and lyophilization parameters, methodological safeguards, and systematic safety assessments was incomplete in many studies. Despite the broad multi-branch search strategy, heterogeneous and evolving terminology for MSC-derived products and lyophilization may have resulted in some relevant studies not being identified. Eligibility was restricted to English-language full-text publications, which may have excluded otherwise relevant evidence published in other languages. These limitations constrain cross-study comparability

and clinical generalizability and should be considered when interpreting the predominantly favorable preclinical findings.

Further development should focus on reproducible and well-characterized lyophilized MSC-derived products suitable for clinical evaluation. The substantial variability in product preparation, formulation, and lyophilization procedures observed in this review, together with incomplete reporting of manufacturing parameters, highlights the need for standardized processes and assessment of batch-to-batch consistency. The inherent heterogeneity of the parental MSC populations represents an additional challenge for product standardization [65]. Particular attention should be given to raw-material control, sterility and endotoxin testing, formulation-specific residual moisture limits, reconstitution consistency, and validated functional potency assays. For EV-based products, these priorities are consistent with MISEV2023 recommendations on reporting source and processing variables, product characterization, storage conditions, and biological activity [54]. The differences in product composition and biological characteristics identified in this review also have implications for quality control and regulatory assessment. While EV-based products require vesicle-specific characterization and secretome preparations require assessment of their complex biological composition [54], non-viable cellular materials and synthetic formulations may require different product-specific quality attributes. Accordingly, regulatory pathways and quality-control specifications should reflect the characteristics and intended use of each product rather than lyophilization status alone. Studies should distinguish explicitly between three related but separate questions: whether structural and functional properties are retained after lyophilization, whether the lyophilized product remains stable during defined storage periods, and whether the resulting lyophilized product demonstrates therapeutic effects in vivo. Direct comparisons between otherwise comparable fresh or non-lyophilized and lyophilized preparations, incorporating both physicochemical characterization and functional potency assays, are needed to establish preservation rather than post-lyophilization activity alone. Shelf-life claims should be supported by longitudinal, product-specific stability studies that include biological activity or potency where relevant, consistent with established principles for stability testing of biological products [57]. Preclinical studies should incorporate the methodological safeguards identified by ARRIVE 2.0, particularly appropriate randomization, blinding, sample-size justification, and transparent reporting [62]. Before broader clinical evaluation, standardized manufacturing and characterization should be accompanied by validated potency and stability assessments, systematic toxicological and safety evaluation, and rational dose selection. Early-phase human trials should incorporate prospectively defined safety monitoring, including adverse events, laboratory toxicity, immunogenicity, dose-related effects, and sufficiently long follow-up, together with clinically meaningful efficacy endpoints.

5. Conclusions

Current evidence indicates that biologically relevant properties can be retained after lyophilization in some MSC-derived products and that lyophilized preparations can remain therapeutically active, although the extent of preservation varies across products and formulations. Therapeutic activity was reported across diverse regenerative models, but the evidence remains predominantly preclinical and methodologically heterogeneous, with only one study including a human interventional component. These findings support proof of concept rather than clinical readiness. No major systemic toxicity signal was identified in the available evidence, but systematic safety assessment was limited and human safety data were restricted to a single study. Post-lyophilization biological activity should not be considered equivalent to demonstrated preservation unless supported by an appropriate fresh or non-lyophilized comparator, and reported storage conditions alone do not establish

product stability. Clinical translation of lyophilized MSC-derived products will require standardized manufacturing and characterization, validated measures of post-lyophilization potency and storage stability, systematic safety assessment, and confirmation of therapeutic efficacy in well-designed human studies. The available evidence therefore supports further product development and clinical investigation but does not establish readiness for routine clinical use.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ph19111837/s1, Table S1: Database-specific search strategies. Table S2: Full-text reports excluded after eligibility assessment [66–72] and reasons for exclusion. Table S3: Product characteristics, lyophilization procedures, and formulation of MSC-derived interventions. Table S4: Numerical therapeutic-effect results. Table S5: Risk-of-bias assessments and supporting explanations.

Author Contributions: Conceptualization, B.S. and I.F.; methodology, B.S. and I.F.; validation, B.S., A.B., A.G., M.K., A.A., A.Z., B.A. and I.F.; formal analysis, B.S., A.B., A.G., M.K., A.A., A.Z., B.A. and I.F.; investigation, B.S., A.B., A.G., M.K., A.A., A.Z., B.A. and I.F.; data curation, B.S., A.B., A.G., M.K., A.A., A.Z., B.A. and I.F.; writing—original draft preparation, B.S., A.B., A.G., M.K., A.A., A.Z., B.A. and I.F.; writing—review and editing, B.S., A.B., A.G., M.K., A.A., A.Z., B.A. and I.F.; visualization, B.S. and I.F.; supervision, I.F.; project administration, B.S. and I.F. All authors have read and agreed to the published version of the manuscript.

Funding: The study and manuscript were prepared within the framework of the grant-funded project BR28713159 "Innovative approaches to the development of a cell-based product for the therapy of chronic and socially significant diseases". Funding sources of included reports were not systematically charted. The grant supported the project; review design, screening, charting, synthesis, interpretation, and manuscript preparation were undertaken by the authors.

Institutional Review Board Statement: Not applicable. This review used published data and did not involve new recruitment of human participants or identifiable individual-level data.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting the findings of this review are available within the article and its Supplementary Materials.

Acknowledgments: The authors express their gratitude for the administrative and technical support provided by the S.D. Asfendiyarov Kazakh National Medical University.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

2D	Two-dimensional
3D	Three-dimensional
ADSC	Adipose-derived stem cell
ADSCC-CM	Adipose-derived stem cell concentrated conditioned medium
ALF	Acute liver failure
ALIX	Apoptosis-linked gene 2-interacting protein X
ALP	Alkaline phosphatase
apoVs	Apoptotic vesicles
apoV-HS	Apoptotic vesicle-containing adhesive hydrogel sponge
ARDS, ADSCs	Acute respiratory distress syndrome
ARRIVE	Animal Research: Reporting of In Vivo Experiments
ASC-CM	Adipose-derived stem cell-conditioned medium
BM-MSC, BMSC	Bone marrow-derived mesenchymal stromal cell
BM-MSC-CM, BMSC-CM	Bone marrow-derived mesenchymal stromal cell-conditioned medium

BMSC-EVs	Bone marrow-derived mesenchymal stromal cell-derived extracellular vesicles
BMSC-OI-exo	Osteoinductive exosomes derived from bone marrow mesenchymal stromal cells
BMP2	Bone morphogenetic protein 2
bUC-MSCs	Bovine umbilical cord-derived mesenchymal stromal cells
CCK-8	Cell Counting Kit-8
CD3	Cluster of differentiation 3
CD9	Cluster of differentiation 9
CD81	Cluster of differentiation 81
CM	Conditioned medium
CM-FD	Conditioned medium immobilized on a matrix by freeze-drying
CM-LYO	Conditioned medium-loaded collagen membrane prepared by lyophilization
CM-SOAK	Collagen membrane prepared by soaking in conditioned medium
CM0	Conditioned medium lyophilized without a cryoprotectant
CM1	Conditioned medium lyophilized with trehalose
CM2	Conditioned medium lyophilized with trehalose and PVP40
CM10	Adipose-derived stem cell-conditioned medium concentrated tenfold
cMSC-CM	Corneal mesenchymal stromal cell-conditioned medium
COVID-19	Coronavirus disease 2019
CTX	Cyclophosphamide
DF-MSC, DF-MSCs	Dental follicle-derived mesenchymal stromal cells
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPSC-CM	Dental pulp stem cell-conditioned medium
DSS	Dextran sodium sulfate
ECM	Extracellular matrix
EdU	5-Ethynyl-2′-deoxyuridine
EV, EVs	Extracellular vesicle, extracellular vesicles
FBMSC-CMM	Freeze-dried bone marrow-derived mesenchymal stromal cell-conditioned medium membrane
FDSG	Freeze-dried secretome gel
Ga/CSSF	Gallium-containing chitosan/silk fibroin sponge scaffold
Ga/CSSF-Exo	Exosome-loaded gallium-containing chitosan/silk fibroin sponge scaffold
HA/CS	Hyaluronic acid/chondroitin sulfate
hBMSC-CM	Human bone marrow-derived mesenchymal stromal cell-conditioned medium
HD-DP7	Hyaluronic acid hydrogel coated with polydopamine and antimicrobial peptide DP7
HD-DP7/Exo	Exosome-loaded HD-DP7 hydrogel
HDFs	Human dermal fibroblasts
HFS	Human fetal mesenchymal stromal cell secretome
HFS-PLGA	Human fetal mesenchymal stromal cell secretome encapsulated in PLGA
HPMSCs	Human placenta-derived mesenchymal stromal cells
hUC-MSC, HucMSC	Human umbilical cord-derived mesenchymal stromal cell
HUVEC	Human umbilical vein endothelial cell
LPS	Lipopolysaccharide
Lyo-CM	Lyophilized conditioned medium
MBG	Mesoporous bioactive glass
MEDLINE	Medical Literature Analysis and Retrieval System Online
MI	Myocardial infarction
miRNA, miRNAs	MicroRNA, microRNAs
MISEV2023	Minimal information for studies of extracellular vesicles 2023
MSC, MSCs	Mesenchymal stromal cell, mesenchymal stromal cells
MSC-apoV, MSC-apoVs	Mesenchymal stromal cell-derived apoptotic vesicle, apoptotic vesicles
MSC-CM	Mesenchymal stromal cell-conditioned medium
MSC-EV, MSC-EVs	Mesenchymal stromal cell-derived extracellular vesicle, extracellular vesicles
MSC-L	Mesenchymal stromal cell lyophilizate
MSC-S	Mesenchymal stromal cell secretome

MSC-SLP	Mesenchymal stromal cell supernatant lyophilized powder
NR	Not reported
NS	Not statistically significant
PBS	Phosphate-buffered saline
PDGF-BB	Platelet-derived growth factor BB
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(L-lactic acid)
PRF	Platelet-rich fibrin
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
PRP	Platelet-rich plasma
PS	Phosphatidylserine
PVP40	Polyvinylpyrrolidone with an average molecular mass of approximately 40 kDa
rhBMP2	Recombinant human bone morphogenetic protein 2
RNA	Ribonucleic acid
RoB 1	Original Cochrane risk-of-bias tool
RoB 2	Revised Cochrane risk-of-bias tool for randomized trials
ROBINS-I	Risk Of Bias In Non-randomized Studies of Interventions
RT	Room temperature
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SFM	Serum-free medium
sMSC	Mesenchymal stromal cell-derived secretome
ST	Secretome
ST-CS-MS	Secretome-loaded chitosan microspheres
STZ	Streptozotocin
synMSC, synMSCs	Synthetic mesenchymal stromal cell particles
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
TF	Tissue factor
TNF-α	Tumor necrosis factor alpha
UC-MSC	Umbilical cord-derived mesenchymal stromal cell
VEGF	Vascular endothelial growth factor
WJ-MSC	Wharton's jelly-derived mesenchymal stromal cell

References

1. Trigo, C.M.; Rodrigues, J.S.; Camões, S.P.; Solá, S.; Miranda, J.P. Mesenchymal Stem Cell Secretome for Regenerative Medicine: Where Do We Stand? *J. Adv. Res.* **2025**, *70*, 103–124. [CrossRef] [PubMed]
2. Bogatcheva, N.V.; Coleman, M.E. Conditioned Medium of Mesenchymal Stromal Cells: A New Class of Therapeutics. *Biochemistry* **2019**, *84*, 1375–1389. [CrossRef] [PubMed]
3. Golan, M.E.; Stice, S.L. Extracellular Vesicle Lyophilization for Enhanced Distribution to the Point of Care. *Extracell. Vesicle* **2024**, *3*, 100041. [CrossRef]
4. Ahmadian, S.; Jafari, N.; Tamadon, A.; Ghaffarzadeh, A.; Rahbarghazi, R.; Mahdipour, M. Different Storage and Freezing Protocols for Extracellular Vesicles: A Systematic Review. *Stem Cell Res. Ther.* **2024**, *15*, 453. [CrossRef] [PubMed]
5. Abla, K.K.; Mehanna, M.M. Freeze-Drying: A Flourishing Strategy to Fabricate Stable Pharmaceutical and Biological Products. *Int. J. Pharm.* **2022**, *628*, 122233. [CrossRef] [PubMed]
6. Kommineni, N.; Butreddy, A.; Sainaga Jyothi, V.G.S.; Angsantikul, P. Freeze-Drying for the Preservation of Immunoengineering Products. *iScience* **2022**, *25*, 105127. [CrossRef] [PubMed]
7. Ramakrishnan, P.; Jalaludeen, A.M.; Vinayagam, S.; Gnanasekaran, L.; Durairaj, T.; Rajamohan, R.; Sundaram, T. Mesenchymal Stromal Cell Secretome in Scaffold-Based Drug Delivery: Advances, Applications, and Future Directions. *Int. J. Biol. Macromol.* **2025**, *329*, 147919. [CrossRef] [PubMed]
8. Paresishvili, T.; Kakabadze, Z. Freeze-Dried Mesenchymal Stem Cells: From Bench to Bedside. *Rev. Adv. Biol.* **2024**, *8*, e2300155. [CrossRef] [PubMed]
9. Suhandi, C.; Mohammed, A.F.A.; Wilar, G.; El-Rayyes, A.; Wathoni, N. Effectiveness of Mesenchymal Stem Cell Secretome on Wound Healing: A Systematic Review and Meta-Analysis. *Tissue Eng. Regen. Med.* **2023**, *20*, 1053–1062. [CrossRef] [PubMed]
10. Lu, Z.; Chen, H.; Chang, H.; Xiong, Y.; Miao, X.; Zhang, C.; Wu, Q.; Sun, Y.; Feng, L.; Hao, Y.; et al. Therapeutic Effects of Mesenchymal Stem Cells and Their Secretomes (Extracellular Vesicles) with Bone-Implant Scaffolds for Bone Regeneration: A Systematic Review and Network Meta-Analysis of Preclinical Studies. *Acta Biomater.* **2026**, *213*, 88–102. [CrossRef] [PubMed]

11. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* **2021**, *372*, n71. [CrossRef] [PubMed]
12. Hooijmans, C.R.; Rovers, M.M.; de Vries, R.B.M.; Leenaars, M.; Ritskes-Hoitinga, M.; Langendam, M.W. SYRCLE's Risk of Bias Tool for Animal Studies. *BMC Med. Res. Methodol.* **2014**, *14*, 43. [CrossRef] [PubMed]
13. Sterne, J.A.C.; Savović, J.; Page, M.J.; Elbers, R.G.; Blencowe, N.S.; Boutron, I.; Cates, C.J.; Cheng, H.-Y.; Corbett, M.S.; Eldridge, S.M.; et al. RoB 2: A Revised Tool for Assessing Risk of Bias in Randomised Trials. *BMJ* **2019**, *366*, l4898. [CrossRef] [PubMed]
14. Sterne, J.A.C.; Hernán, M.A.; Reeves, B.C.; Savović, J.; Berkman, N.D.; Viswanathan, M.; Henry, D.; Altman, D.G.; Ansari, M.T.; Boutron, I.; et al. ROBINS-I: A Tool for Assessing Risk of Bias in Non-Randomised Studies of Interventions. *BMJ* **2016**, *355*, i4919. [CrossRef] [PubMed]
15. Jiang, Y.; Li, X.; Huang, R.; Lei, F.; Li, L.; Yang, B.; Zen, W.; Tan, H.; Huang, Y.; Hu, J.; et al. Lyophilized Apoptotic Vesicles Improve Hemostasis and Bone Regeneration in Traumatic Patients with Impacted Third Molar Extraction. *Mol. Ther.* **2025**, *33*, 2931–2944. [CrossRef] [PubMed]
16. Díaz, C.; Frankenberg, D.; Retamal, P.; Escobar, C.; Morera, F.J.; Paiva, L.; Leiva, B.; Quiroga, B.; Peralta, O.A. Evaluation of an Experimental Therapy Based on Bovine Mesenchymal Stem Cell (MSC) Secretome for the Treatment of Endometritis in Dairy Cows. *Sci. Rep.* **2025**, *15*, 43626. [CrossRef] [PubMed]
17. Berni, P.; Del Bue, M.; Conti, V.; Andreoli, V.; Ramoni, R.; Angelone, M.; Squassino, G.P.; Bari, E.; Torre, M.L.; Rinaldi, M.; et al. Clinical Evaluation of Freeze-Dried Secretome (Lyosecretome) for Osteoarthritis: A Controlled Trial in Dogs and Preliminary Safety Assessment in Horses. *Int. J. Pharm.* **2025**, *681*, 125864. [CrossRef] [PubMed]
18. Mocchi, M.; Bari, E.; Dotti, S.; Villa, R.; Berni, P.; Conti, V.; Del Bue, M.; Squassino, G.P.; Segale, L.; Ramoni, R.; et al. Canine Mesenchymal Cell Lyosecretome Production and Safety Evaluation after Allogenic Intraarticular Injection in Osteoarthritic Dogs. *Animals* **2021**, *11*, 3271. [CrossRef] [PubMed]
19. Freitas, N.P.P.; Silva, B.D.P.; Bezerra, M.R.L.; Pescini, L.Y.G.; Olinda, R.G.; Salgueiro, C.C.d.M.; Nunes, J.F.; Martins, J.A.M.; Neto, S.G.; Martins, L.T. Freeze-Dried Platelet-Rich Plasma and Stem Cell-Conditioned Medium for Therapeutic Use in Horses. *J. Equine Vet. Sci.* **2023**, *121*, 104189. [CrossRef] [PubMed]
20. Mahesh, A.K.D.; Namasudra, M.; Manna, C.; Pradhan, S.; Hansda, R.N.; Bag, S.; Mondal, S.; Das, K. Bovine Umbilical Cord Tissue-derived MSCs and Its Conditioned Medium Promote the Healing of Excisional Wound in Rats. *Regen. Med.* **2026**, *21*, 393–404. [CrossRef] [PubMed]
21. Chen, X.; Li, B.; Gu, W.; Li, M.; Zhang, Z. Advanced Lyophilized Mesenchymal Stem Cell-Conditioned Medium Formulation Improves Therapeutic Efficacy in Skin Wound Repair. *Regen. Biomater.* **2026**, *13*, rbag140. [CrossRef] [PubMed]
22. Zhang, X.; Shi, W.; Wang, X.; Zou, Y.; Xiang, W.; Lu, N. Evaluation of the Composite Skin Patch Loaded with Bioactive Functional Factors Derived from Multicellular Spheres of EMSCs for Regeneration of Full-Thickness Skin Defects in Rats. *Curr. Stem Cell Res. Ther.* **2024**, *19*, 1142–1152. [CrossRef] [PubMed]
23. Wang, B.; Pang, M.; Song, Y.; Wang, H.; Qi, P.; Bai, S.; Lei, X.; Wei, S.; Zong, Z.; Lin, S.; et al. Human Fetal Mesenchymal Stem Cells Secretome Promotes Scarless Diabetic Wound Healing through Heat-Shock Protein Family. *Bioeng. Transl. Med.* **2023**, *8*, e10354. [CrossRef] [PubMed]
24. Peng, Y.; Xuan, M.; Zou, J.; Liu, H.; Zhuo, Z.; Wan, Y.; Cheng, B. Freeze-Dried Rat Bone Marrow Mesenchymal Stem Cell Paracrine Factors: A Simplified Novel Material for Skin Wound Therapy. *Tissue Eng. Part A* **2015**, *21*, 1036–1046. [CrossRef] [PubMed]
25. Sun, B.; Guo, S.; Xu, F.; Wang, B.; Liu, X.; Zhang, Y.; Xu, Y. Concentrated Hypoxia-Preconditioned Adipose Mesenchymal Stem Cell-Conditioned Medium Improves Wounds Healing in Full-Thickness Skin Defect Model. *Int. Sch. Res. Not.* **2014**, *2014*, 652713. [CrossRef] [PubMed]
26. Shi, X.; Li, Y.; Kang, S.; Zhao, X.; Liu, L.; Yuan, F.; He, L.; Lu, H.; Liu, J. Dual-Functional Gallium/Chitosan/Silk/Umbilical Cord Mesenchymal Stem Cell Exosome Sponge Scaffold for Diabetic Wound by Angiogenesis and Antibacteria. *Int. J. Biol. Macromol.* **2024**, *274*, 133420. [CrossRef] [PubMed]
27. Yang, Y.; Zhang, J.; Wu, S.; Deng, Y.; Wang, S.; Xie, L.; Li, X.; Yang, L. Exosome/Antimicrobial Peptide Laden Hydrogel Wound Dressings Promote Scarless Wound Healing through miR-21-5p-Mediated Multiple Functions. *Biomaterials* **2024**, *308*, 122558. [CrossRef] [PubMed]
28. Bulut, O.; Genc, D.; Demirci, Ç.E.; Tekin, L.; Dümbek, T.M.; Bülbül, A. Regenerative Effect of Lyophilized Dental Follicle Mesenchymal Stem Cells and Platelet-Rich Fibrin in Skin Wounds in Geriatric and Young Rats. *Sci. Rep.* **2025**, *15*, 6623. [CrossRef] [PubMed]
29. Hendrawan, S.; Marcelina, O.; Tan, S.T.; Baer, H.U. Immobilization of hUC-MSCs Conditioned Medium on 3D PLLA Collagen-Coated Matrix Enhances Diabetic Wound Healing Progression. *Eng. Regen.* **2024**, *5*, 421–431. [CrossRef]
30. Widowati, W.; Hidayat, M.; Faried, A.; Rahardja, F.; Gunanegara, R.F.; Triharsiwi, D.N.; Basyasyah, R.D.; Azis, R.; Priyandoko, D.; Hadiprasetyo, D.S.; et al. Efficacy of Freeze-Dried Human Wharton's Jelly Mesenchymal Stem Cell Secretome Gel Toward Wound Healing Rat Model. *Jordan J. Biol. Sci.* **2026**, *19*, 159–168. [CrossRef]

31. Duan, W.; Wang, H.; Wang, Z.; Ren, Z.; Li, X.; He, F.; Li, S.; Guan, Y.; Liu, F.; Chen, L.; et al. Multi-Functional Composite Dressings with Sustained Release of MSC-SLP and Anti-Adhesion Property for Accelerating Wound Healing. *Mater. Today Bio* **2024**, *25*, 100979. [CrossRef] [PubMed]
32. Joseph, A.; Baiju, I.; Bhat, I.A.; Pandey, S.; Bharti, M.; Verma, M.; Pratap Singh, A.; Ansari, M.M.; Chandra, V.; Saikumar, G.; et al. Mesenchymal Stem Cell-Conditioned Media: A Novel Alternative of Stem Cell Therapy for Quality Wound Healing. *J. Cell. Physiol.* **2020**, *235*, 5555–5569. [CrossRef] [PubMed]
33. Zhang, C.; Wang, T.; Zhang, L.; Chen, P.; Tang, S.; Chen, A.; Li, M.; Peng, G.; Gao, H.; Weng, H.; et al. Combination of Lyophilized Adipose-Derived Stem Cell Concentrated Conditioned Medium and Polysaccharide Hydrogel in the Inhibition of Hypertrophic Scarring. *Stem Cell Res. Ther.* **2021**, *12*, 23. [CrossRef] [PubMed]
34. Tutak, F.N.; Kıvrak, E.G. The Effect of Human Umbilical Cord-Derived Lyophilized Stem Cells on Fat Graft Viability: An Experimental Study. *Aesthetic Plast. Surg.* **2022**, *46*, 1973–1982. [CrossRef] [PubMed]
35. Kanazawa, S.; Ono, S.; Shinchi, H.; Kanno, H.; Ueda, K.; Ishimaru, N.; Takemoto, K.; Hoshi, K. Dental Pulp Cell-Derived Conditioned Culture Media Promotes Periodontal Repair through Immunomodulation and Bone Regeneration. *Regen. Ther.* **2026**, *33*, 101144. [CrossRef] [PubMed]
36. Buss, L.F.; de Martin, G.S.; Martinez, E.F.; Filgueiras, I.A.d.A.A.P.; Magnabosco, J.L.; Alves, B.F.; de Macedo Almeida, B.; Kotaka, T.; Teixeira, M.L.; Ferreira, J.R.M.; et al. Conditioned Media from Human Pulp Stem Cell Cultures Improve Bone Regeneration in Rat Calvarial Critical-Size Defects. *J. Funct. Biomater.* **2023**, *14*, 396. [CrossRef] [PubMed]
37. Shanbhag, S.; Kampleitner, C.; Al-Sharabi, N.; Mohamed-Ahmed, S.; Apaza Alccayhuaman, K.A.; Heimel, P.; Tangl, S.; Beinlich, A.; Rana, N.; Sanz, M.; et al. Functionalizing Collagen Membranes with MSC-Conditioned Media Promotes Guided Bone Regeneration in Rat Calvarial Defects. *Cells* **2023**, *12*, 767. [CrossRef] [PubMed]
38. Huang, C.-C.; Kang, M.; Debnath, K.; Leung, K.; Raghavan, V.; Lu, Y.; Cooper, L.F.; Gajendrareddy, P.; Ravindran, S. Functionality of Lyophilized Osteoinductive EVs: A Mechanistic Study. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1452428. [CrossRef] [PubMed]
39. Liu, A.; Lin, D.; Zhao, H.; Chen, L.; Cai, B.; Lin, K.; Shen, S.G.F. Optimized BMSC-Derived Osteoinductive Exosomes Immobilized in Hierarchical Scaffold via Lyophilization for Bone Repair through Bmpr2/Acvr2b Competitive Receptor-Activated Smad Pathway. *Biomaterials* **2021**, *272*, 120718. [CrossRef] [PubMed]
40. Jabbehdari, S.; Yazdanpanah, G.; Kanu, L.N.; Chen, E.; Kang, K.; Anwar, K.N.; Ghassemi, M.; Hematti, P.; Rosenblatt, M.I.; Djalilian, A.R. Therapeutic Effects of Lyophilized Conditioned-Medium Derived from Corneal Mesenchymal Stromal Cells on Corneal Epithelial Wound Healing. *Curr. Eye Res.* **2020**, *45*, 1490–1496. [CrossRef] [PubMed]
41. Fernandes-Cunha, G.M.; Na, K.-S.; Putra, I.; Lee, H.J.; Hull, S.; Cheng, Y.-C.; Blanco, I.J.; Eslani, M.; Djalilian, A.R.; Myung, D. Corneal Wound Healing Effects of Mesenchymal Stem Cell Secretome Delivered Within a Viscoelastic Gel Carrier. *Stem Cells Transl. Med.* **2019**, *8*, 478–489. [CrossRef] [PubMed]
42. Bahr, M.M.; Amer, M.S.; Abo-EL-Sooud, K.; Kamel, S.M.; Fouly, M.A.; Abdallah, A.N.; Shamaa, A.A.; El-Tookhy, O.S. Effect of Topical Application of Lyophilized Xenogenous Mesenchymal Stem Cell-Derived Extracellular Vesicles on Central and Peripheral Corneal Ulcers Healing in Rabbits. *Adv. Anim. Vet. Sci.* **2023**, *11*, 132–140. [CrossRef]
43. Yang, S.; Chen, S.; Zhuang, W.; Wang, Q.; Xu, Y.; Zhan, T.; Huang, W.; Tan, Y. Lyophilized Mesenchymal Stromal Cell-Derived Extracellular Vesicles for the Oral Treatment of Inflammatory Bowel Disease: A Novel Cell-Free Therapeutic Strategy. *Stem Cell Res. Ther.* **2026**, *17*, 213. [CrossRef] [PubMed]
44. Oh, S.-J.; Nguyen, T.T.; Seo, Y.; Park, H.-J.; Ahn, J.-S.; Shin, Y.Y.; Kang, B.-J.; Jang, M.; Park, J.; Jeong, J.-H.; et al. Sustained Release of Stem Cell Secretome from Nano-Villi Chitosan Microspheres for Effective Treatment of Atopic Dermatitis. *Int. J. Biol. Macromol.* **2024**, *277*, 134344. [CrossRef] [PubMed]
45. Kudinov, V.A.; Artyushev, R.I.; Zurina, I.M.; Zorina, E.S.; Lapshin, R.D.; Snopova, L.B.; Mukhina, I.V.; Saburina, I.N. Inhaled Placental Mesenchymal Stromal Cell Secretome from Two- and Three-Dimensional Cell Cultures Promotes Survival and Regeneration in Acute Lung Injury Model in Mice. *Int. J. Mol. Sci.* **2022**, *23*, 3417. [CrossRef] [PubMed]
46. Peng, W.; Chang, M.; Wu, Y.; Zhu, W.; Tong, L.; Zhang, G.; Wang, Q.; Liu, J.; Zhu, X.; Cheng, T.; et al. Lyophilized Powder of Mesenchymal Stem Cell Supernatant Attenuates Acute Lung Injury through the IL-6-p-STAT3-p63-JAG2 Pathway. *Stem Cell Res. Ther.* **2021**, *12*, 216. [CrossRef] [PubMed]
47. Suzuki, F.; Haga, H.; Inuzuka, T.; Hoshikawa, K.; Katsumi, T.; Maki, K.; Uchiyama, F.; Ueno, Y. Lyophilized Mesenchymal Stem Cell-Derived Extracellular Vesicles Improve Outcomes in Acute Liver Failure Models. *Am. J. Physiol. Cell Physiol.* **2026**, *330*, C540–C551. [CrossRef] [PubMed]
48. Yang, B.; Luo, H.; Yan, X.; Li, L.; Huang, Z.; Jiang, Y.; Mao, X.; Shi, S.; Ma, L. Lyophilized Apoptotic Vesicles Restore DNA Damage and Mitochondria Dysfunction to Ameliorate Radiation Enteritis. *J. Nanobiotechnol.* **2025**, *23*, 521. [CrossRef] [PubMed]
49. Ebrahim, N.; Al Saihati, H.A.; Alali, Z.; Mahmoud, S.Y.M.; Rabaan, A.A.; Dessouky, A.A.; Salim, R.F.; Shamaa, A.A.; Abdallah, A.N.; Elsherbiny, N.M.; et al. Lyophilized MSC-EVs Attenuates COVID-19 Pathogenesis by Regulating the JAK/STAT Pathway. *Stem Cell Res. Ther.* **2025**, *16*, 244. [CrossRef] [PubMed]

50. Kakabadze, Z.; Kipshidze, N.; Paresishvili, T.; Kipshidze, N.; Vadachkoria, Z.; Chakhunashvili, D. Human Placental Mesenchymal Stem Cells for the Treatment of ARDS in Rat. *Stem Cells Int.* **2022**, *2022*, 8418509. [CrossRef] [PubMed]
51. Zhang, Y.; Li, D.; Han, Y.; Wu, M.; Zhang, S.; Ma, H.; Liu, L.; Ju, X. Intraovarian Injection of 3D-MSC-EVs-ECM Gel Significantly Improved Rat Ovarian Function after Chemotherapy. *Reprod. Biol. Endocrinol.* **2024**, *22*, 125. [CrossRef] [PubMed]
52. Jiang, Y.; Hao, M.; Jiang, F.; Li, J.; Yang, K.; Li, C.; Ma, L.; Liu, S.; Kou, X.; Shi, S.; et al. Lyophilized Apoptotic Vesicle-Encapsulated Adhesive Hydrogel Sponge as a Rapid Hemostat for Traumatic Hemorrhage in Coagulopathy. *J. Nanobiotechnol.* **2023**, *21*, 407. [CrossRef] [PubMed]
53. Luo, L.; Tang, J.; Nishi, K.; Yan, C.; Dinh, P.-U.; Cores, J.; Kudo, T.; Zhang, J.; Li, T.-S.; Cheng, K. Fabrication of Synthetic Mesenchymal Stem Cells for the Treatment of Acute Myocardial Infarction in Mice. *Circ. Res.* **2017**, *120*, 1768–1775. [CrossRef] [PubMed]
54. Welsh, J.A.; Goberdhan, D.C.I.; O'Driscoll, L.; Buzas, E.I.; Blenkiron, C.; Bussolati, B.; Cai, H.; Di Vizio, D.; Driedonks, T.A.P.; Erdbrügger, U.; et al. Minimal Information for Studies of Extracellular Vesicles (MISEV2023): From Basic to Advanced Approaches. *J. Extracell. Vesicles* **2024**, *13*, e12404. [CrossRef] [PubMed]
55. Baigenzhin, A.; Chuvakova, E.; Zhakupova, A.; Akhayeva, A.; Ganina, A. Extracellular Vesicles and Lyophilization: Getting Functionally Stable Cell-Free Biomedical Products. *J. Clin. Med. Kaz.* **2025**, *22*, 117–125. [CrossRef] [PubMed]
56. Luo, J.; Cui, Y.; Xu, L.; Zhang, J.; Chen, J.; Li, X.; Zeng, B.; Deng, Z.; Shao, L. Layered double hydroxides for regenerative nanomedicine and tissue engineering: Recent advances and future perspectives. *J. Nanobiotechnol.* **2025**, *23*, 370. [CrossRef] [PubMed]
57. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). ICH Harmonised Tripartite Guideline: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (Q5C). 1995. Available online: https://database.ich.org/sites/default/files/Q5C_Guideline.pdf (accessed on 6 October 2026).
58. Van Delen, M.; Derdelinckx, J.; Wouters, K.; Nelissen, I.; Cools, N. A Systematic Review and Meta-Analysis of Clinical Trials Assessing Safety and Efficacy of Human Extracellular Vesicle-Based Therapy. *J. Extracell. Vesicles* **2024**, *13*, e12458. [CrossRef] [PubMed]
59. Montero-Vilchez, T.; Sierra-Sánchez, Á.; Sanchez-Diaz, M.; Quiñones-Vico, M.I.; Sanabria-de-la-Torre, R.; Martinez-Lopez, A.; Arias-Santiago, S. Mesenchymal Stromal Cell-Conditioned Medium for Skin Diseases: A Systematic Review. *Front. Cell Dev. Biol.* **2021**, *9*, 654210. [CrossRef] [PubMed]
60. Mussin, N.M.; Zhilisbayeva, K.R.; Baspakova, A.; Kurmanalina, M.A.; Tamadon, A. Umbrella Review of Mesenchymal Stem Cell-Derived Extracellular Vesicles in Preclinical Models: Therapeutic Efficacy across Diverse Conditions. *Front. Cell Dev. Biol.* **2025**, *13*, 1655623. [CrossRef] [PubMed]
61. Wang, Y.; Zhu, J.; Ma, Q.; Zhou, W.; Yang, L.; Sheng, S.; Zhu, F.; Xia, Z. Trends in Mesenchymal Stem Cell-Derived Extracellular Vesicles Clinical Trials 2014–2024: Is Efficacy Optimal in a Narrow Dose Range? *Front. Med.* **2025**, *12*, 1625787. [CrossRef] [PubMed]
62. Percie du Sert, N.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; et al. The ARRIVE Guidelines 2.0: Updated Guidelines for Reporting Animal Research. *PLoS Biol.* **2020**, *18*, e3000410. [CrossRef] [PubMed]
63. Archambault, J.; Moreira, A.; McDaniel, D.; Winter, L.; Sun, L.; Hornsby, P. Therapeutic Potential of Mesenchymal Stromal Cells for Hypoxic Ischemic Encephalopathy: A Systematic Review and Meta-Analysis of Preclinical Studies. *PLoS ONE* **2017**, *12*, e0189895. [CrossRef] [PubMed]
64. Hirst, J.A.; Howick, J.; Aronson, J.K.; Roberts, N.; Perera, R.; Koshiaris, C.; Heneghan, C. The Need for Randomization in Animal Trials: An Overview of Systematic Reviews. *PLoS ONE* **2014**, *9*, e98856. [CrossRef] [PubMed]
65. Lyu, Z.; Xin, M.; Oyston, D.R.; Xue, T.; Kang, H.; Wang, X.; Wang, Z.; Li, Q. Cause and consequence of heterogeneity in human mesenchymal stem cells: Challenges in clinical application. *Pathol. Res. Pract.* **2024**, *260*, 155354. [CrossRef] [PubMed]
66. Munoz-Perez, E.; Santos-Vizcaino, E.; Goyanes, A.; Basit, A.W.; Hernandez, R.M. 3D-Printed Core-Shell Tablet for Effective Oral Delivery of AT-MSC Secretome in Inflammatory Bowel Disease Therapy. *Drug Deliv. Transl. Res.* **2026**, *16*, 2179–2193. [CrossRef] [PubMed]
67. Widowati, W.; Faried, A.; Gunanegara, R.F.; Rahardja, F.; Zahiroh, F.H.; Sutendi, A.F.; Nindya, F.S.; Azis, R.; Ekajaya, R.K. The Potential of Human Wharton's Jelly Mesenchymal Stem Cells Secretome Based Topical Gel for Therapeutic Application. *Avicenna J. Med. Biotechnol.* **2024**, *16*, 233–243. [CrossRef] [PubMed]
68. Nainggolan, A.D.C.; Hartrianti, P.; Anjani, Q.K.; Donnelly, R.F.; Putra, A.B.N.; Kho, K.; Kurniawan, A.; Andranilla, R.K.; Rattu, S.A.; Ramadon, D. Double-Layer Dissolving Microneedles for Delivery of Mesenchymal Stem Cell Secretome: Formulation, Characterisation and Skin Irritation Study. *Eur. J. Pharm. Biopharm.* **2024**, *204*, 114495. [CrossRef] [PubMed]
69. Yao, Y.; Huang, C.; Gu, P.; Wen, T. Combined MSC-Secreted Factors and Neural Stem Cell Transplantation Promote Functional Recovery of PD Rats. *Cell Transplant.* **2016**, *25*, 1101–1113. [CrossRef] [PubMed]

70. Widowati, W.; Faried, A.; Gunanegara, R.F.; Rahardja, F.; Zahiroh, F.H.; Sutendi, A.F.; Nindya, F.S.; Azis, R.; Ekajaya, R.K.; Hadiprasetyo, D.S. Wound Healing Potent of Lyophilized-Secretome Gel from Human Wharton's Jelly Mesenchymal Stem Cells. *Trends Sci.* **2024**, *21*, 8028. [CrossRef]
71. Chen, D.; Xue, Y.; Tang, Y.; Fei, X.M.; Zhuang, Q.; Zhou, W.W.; Lyu, D.M.; Shi, W.T.; Zhang, Z.J.; Zheng, W.J.; et al. Ecto-Mesenchymal Stem Cell-Conditioned Medium Lyophilized Powder Combined with Fibrin Glue to Repair Skin Injury in Rats. *Chin. J. Tissue Eng. Res.* **2023**, *27*, 2350–2355. [CrossRef]
72. Monakova, A.O.; Balabanyan, V.Y.; Vavilova, V.A.; Basalova, N.A.; Popov, V.S.; Akopyan, Z.A.; Efimenko, A.Y. Safety Profile Evaluation of an Original Mesenchymal Stromal Cell Secretome-Based Medicinal Product after Intratesticular Injection in Rabbits. *Saf. Risk Pharmacother.* **2025**, *13*, 213–228. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.