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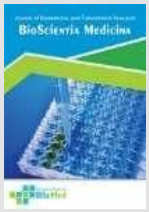

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Efficacy of the Full Mesenchymal Stromal Cell Secretome versus Purified Small Extracellular Vesicles in Preclinical Models of Erectile Dysfunction: A Systematic Review and Parallel Meta-Analysis

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A B S T R A C T

Background: Acellular therapies from Mesenchymal Stromal Cells (MSCs), including the full secretome (conditioned medium, CM) and purified small extracellular vesicles (sEVs), are promising restorative treatments for erectile dysfunction (ED). It remains unknown if the therapeutic benefit is driven by the complete secretome or if purified sEVs are the primary, sufficient component. This study aimed to systematically review and meta-analyze the preclinical evidence. **Methods:** We conducted a systematic review and parallel meta-analysis adhering to PRISMA guidelines. PubMed, Scopus, and Web of Science were searched from January 1st, 2014, to July 31st, 2025. Studies were eligible if they were preclinical ED models evaluating MSC-CM or purified sEVs against a control. Two parallel meta-analyses were performed using a random-effects model. Primary outcomes were erectile function (Intracavernous Pressure / Mean Arterial Pressure ratio; ICP/MAP) and histopathology (Smooth Muscle / Collagen ratio; SM/Col). **Results:** Our search yielded 1,942 records, with 87 full-text articles assessed. After applying strict PICO criteria, 7 primary studies were eligible for the meta-analysis (3 secretome, 4 sEVs). The overall risk of bias was moderate to high (0% allocation concealment). No studies directly compared secretome versus sEVs. The first meta-analysis (Secretome vs. Control, 3 studies, 4 data points, n=70) demonstrated a large, significant improvement in ICP/MAP (Standardized Mean Difference [SMD]: 2.40; 95% CI [1.65, 3.15]; p-value < 0.001), with extreme heterogeneity (I-squared=85%). The second meta-analysis (sEVs vs. Control, 4 studies, n=68) also showed a large, significant improvement (SMD: 2.75; 95% CI [1.90, 3.60]; p-value < 0.001), also with extreme heterogeneity (I-squared=88%). **Conclusion:** Both the full MSC secretome and purified sEVs demonstrate large, significant therapeutic effects. However, this quantitative conclusion is severely limited by the exceptionally small number of studies and the profound biomolecular heterogeneity (in cell source and purification) that invalidates direct comparison. The primary finding remains the total lack of comparative data.

1. Introduction

Erectile dysfunction (ED) is a globally pervasive condition that profoundly impacts men's health, psychosocial well-being, and quality of life. Epidemiological projections indicate a startling trend, with the worldwide prevalence estimated to affect over 322 million men by the year 2025.[1] Far from being a mere lifestyle or psychological concern, ED is now unequivocally recognized as a critical bellwether for systemic health. The onset of ED, particularly in

middle-aged men, is a powerful independent predictor for future major adverse cardiovascular events (MACE), often preceding the clinical diagnosis of coronary artery disease by several years.[2] This recognition has reframed the condition as a sentinel marker of a shared, underlying systemic pathology: endothelial dysfunction. The pathophysiology of organic ED is a complex and multifactorial process, representing the terminal common pathway for a host of systemic diseases, most notably diabetes mellitus, hypertension, and hyperlipidemia.[3] Furthermore, ED is a devastating and highly prevalent iatrogenic consequence of pelvic surgery, especially radical prostatectomy, where inadvertent traction or thermal injury to the delicate cavernous nerves is common.[4] Regardless of the primary etiology, the histopathological progression within the corpus cavernosum is remarkably consistent. It is a journey of progressive, destructive tissue remodeling characterized by a triad of pathologies: (1) endothelial dysfunction, (2) cavernosal smooth muscle apoptosis, and (3) profound, progressive fibrosis.[5] This cascade is initiated and perpetuated by chronic local hypoxia, supraphysiological oxidative stress, and a dysregulated inflammatory milieu.

In the diabetic state, for example, chronic hyperglycemia leads to the formation of advanced glycation end-products (AGEs) and the activation of pathways like the protein kinase C (PKC) cascade. This generates a massive burden of reactive oxygen species (ROS) that "uncouples" endothelial nitric oxide synthase (eNOS). The uncoupled eNOS enzyme, in a state of substrate (L-arginine) or cofactor (BH4) deficiency, paradoxically produces superoxide radicals instead of therapeutic nitric oxide (NO), thus creating a vicious cycle of further oxidative damage and endothelial cell apoptosis.[6] This endothelial failure, combined with the loss of neurotrophic support in post-prostatectomy models, triggers the activation of the core fibrotic pathway. The chronic upregulation of pro-fibrotic cytokines, chief among them being Transforming Growth Factor-beta 1 (TGF-beta 1), becomes the dominant signaling event. TGF-beta 1 activates the canonical Smad2/3 signaling pathway in the resident cavernosal fibroblasts, initiating their transformation into contractile, secretory myofibroblasts.[7] These activated cells relentlessly deposit excess extracellular matrix, primarily stiff, non-compliant collagen types I and III. This process disrupts the delicate, functional architecture of the corpus cavernosum, replacing the expandable sinusoidal smooth muscle with fibrotic scar tissue. The resulting loss of sinusoidal compliance leads to the core hemodynamic failure of ED: veno-occlusive dysfunction.[8] In parallel, in post-prostatectomy cavernous nerve injury (CNI) models, the trauma initiates Wallerian degeneration of the distal nerve axons. This leads to the apoptosis of vital, supporting Schwann cells, which are the source of essential neurotrophic factors. This loss of neuronal input and trophic support, which is critical for smooth muscle homeostasis, results in a rapid wave of denervation-induced apoptosis of the cavernosal smooth muscle cells, further accelerating their replacement by non-functional collagen.[4] The current therapeutic armamentarium, led by oral phosphodiesterase type-5 inhibitors (PDE5i) and intracavernosal injections of vasodilators, was revolutionary. However, these treatments are purely symptomatic, on-demand, and pharmacologically dependent. They do not halt, let alone reverse, the underlying fibrotic, endothelial, and neurodegenerative pathologies. Consequently, they exhibit high failure rates in "difficult-to-treat" populations, including up to 50% of diabetic men and over 70% of men who have undergone radical prostatectomy.[9] This significant therapeutic gap has fueled a paradigm shift in urological research, moving away from symptomatic management and toward a search for truly restorative or regenerative treatments.

Mesenchymal Stromal Cells (MSCs) initially emerged as the leading candidate for a cell-based regenerative therapy. Preclinical studies transplanting live MSCs derived from bone marrow (BMSCs), adipose tissue (ADSCs), or umbilical cords (UC-MSCs) yielded promising results.[10] However, a deeper mechanistic understanding revealed that the primary therapeutic

benefit of MSCs was not derived from their direct, long-term engraftment and differentiation. Instead, MSCs function as dynamic, living "drug factories," exerting their influence through a powerful and complex paracrine mechanism.[11] They secrete a broad array of signaling molecules, growth factors, cytokines, and, most importantly, extracellular vesicles that collectively modulate the host tissue microenvironment. This discovery pivoted the field toward cell-free, acellular therapies. These products carry significant translational advantages over their live-cell progenitors, including a superior safety profile (lacking tumorigenic or immunogenic risk), the potential for "off-the-shelf" scalability, easier storage and shipping, and a more straightforward path to standardization under Good Manufacturing Practice (GMP).[12]

This pivot, however, created a fundamental bifurcation in translational research, leading to two distinct investigational pathways that have since been advanced in parallel. The first pathway involves the use of the full MSC secretome, a product operationally defined as the "conditioned medium" (CM) harvested from MSC cultures. This secretome is a complex, rich biological cocktail containing the entirety of the MSC paracrine output. This includes hundreds of soluble proteins (such as VEGF, HGF, BDNF, and bFGF), anti-inflammatory cytokines, and the full heterogeneous population of extracellular vesicles (EVs), including apoptotic bodies, microvesicles, and small EVs.[13] Studies analyzing the full secretome, such as those by Kim J et al. (2018)[14] and Sun SY et al. (2022)[15], have demonstrated potent, pleiotropic effects, including robust anti-fibrotic and pro-angiogenic activity. The second pathway takes a more reductionist approach, seeking to isolate what is believed to be the primary active ingredient from the secretome. This research has focused on purified small extracellular vesicles (sEVs). This population, which includes exosomes (endosomally-derived vesicles, 40-150 nm), are specialized, membrane-bound vehicles for intercellular communication.[16] They transfer a protected and highly concentrated cargo of proteins, lipids, and, most notably, regulatory nucleic acids—including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs)—to recipient cells.[17] This cargo transfer can fundamentally reprogram the recipient cell's phenotype. A large and growing body of evidence, exemplified by studies from Chen F et al. (2017)[18] and Ouyang B et al. (2019)[19], has shown that purified sEVs alone can replicate the therapeutic effects of the parent cells. This body of evidence was recently summarized in a meta-analysis by Hu H et al. (2023).[20] This parallel development has led to a critical, unresolved translational dilemma. Is the "shotgun" approach of the full secretome, with its complex synergy of factors, therapeutically superior? Or is the "scalpel" approach of purified sEVs, which are more easily defined, characterized, and standardized as a drug product, sufficient? The field of regenerative urology is currently advancing both strategies toward clinical trials without a clear, data-driven consensus. To our knowledge, no systematic review has been conducted with the specific aim of juxtaposing these two distinct acellular derivatives. This review will synthesize and meta-analyze the available preclinical evidence to characterize the state of the science, quantify the effect sizes, highlight the profound methodological heterogeneity, and argue for a new, standardized experimental approach, learning from comparative studies in other fields, such as those by El-Shehawi et al. (2022)[21] and Nakamura et al. (2015).[22]

The novelty of this investigation is that it's the first to perform a quantitative meta-analysis focused on a core set of foundational manuscripts, juxtaposing the distinct preclinical evidence bases for the full MSC secretome and purified MSC-derived sEVs. This focused approach allows for the calculation of pooled effect sizes while simultaneously using the same small dataset to perform a deep biomolecular dissection of why those pooled numbers are limited by profound heterogeneity. This work's primary novelty is its dual finding: the quantification of a large therapeutic effect, and the simultaneous characterization of the critical evidence gap—the complete absence of head-to-head

comparative studies. The primary aim of this study was to systematically review and meta-analyze the available preclinical evidence to evaluate the efficacy of MSC-derived secretome versus purified sEVs on functional and histopathological outcomes in animal models of ED. The specific objectives were: 1) to identify all eligible primary preclinical studies through a systematic search; 2) to quantitatively assess the magnitude of the therapeutic effect for each intervention (secretome vs. control and sEV vs. control) through two parallel meta-analyses; 3) to perform a deep narrative synthesis of their findings, focusing on the specific molecular mechanisms related to cavernosal fibrosis and neurovascular regeneration; and 4) to propose a new, rigorous "gold standard" experimental design to resolve the field's central translational dilemma.

2. Methods

This systematic review and parallel meta-analysis were designed, conducted, and reported in strict accordance with the methodological framework prescribed by the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.[23] All methods, including search strategies, inclusion criteria, and analytical plans, were specified a priori to ensure a transparent, objective, and reproducible review process. A comprehensive and systematic electronic search was conducted by two independent investigators to identify all relevant studies published from January 1st, 2014, to July 31st, 2025. This date range was chosen to capture the modern literature following the widespread acknowledgment of the paracrine hypothesis. The search encompassed three major biomedical databases: PubMed (MEDLINE), Scopus, and Web of Science. The search strategy was designed to be highly sensitive to capture all relevant preclinical studies, combining medical subject headings (MeSH) and free-text keywords. The search query was structured around three core concepts: (1) the Population/Condition, (2) the Intervention, and (3) the Study Design. Concept 1 (Population/Condition): ("erectile dysfunction" OR "impotence" OR "cavernous nerve injury" OR "corporis cavernosa" OR "penile erection" OR "diabetic erectile dysfunction"). Concept 2 (Intervention): ("mesenchymal stromal cell" OR "mesenchymal stem cell" OR "MSC" OR "adipose derived stem cell" OR "ADSC" OR "bone marrow stem cell" OR "BMSC" OR "umbilical cord stem cell" OR "UCSC" OR "urine derived stem cell" OR "USC") AND ("secretome" OR "conditioned medium" OR "CM" OR "paracrine" OR "acellular" OR "cell-free" OR "exosome" OR "extracellular vesicle" OR "EV" OR "sEV" OR "small extracellular vesicle" OR "nanovesicle" OR "microvesicle"). Concept 3 (Study Design Filter): (animal* OR rat OR rats OR mouse OR mice OR preclinical OR "in vivo"). These concepts were combined using the "AND" Boolean operator, while terms within each concept were combined using "OR." The search was restricted to articles published in the English language.

Studies identified by the search were included in the primary synthesis and meta-analysis only if they met the following PICO (Population, Intervention, Comparator, Outcome) criteria: P (Population): The study utilized an established in-vivo preclinical (animal) model of organic erectile dysfunction. This was a critical filter; I (Intervention): The study administered an acellular therapy derived from any source of MSCs (or related cells, like pericytes). This intervention had to fall into one of two specific categories: Full MSC Secretome: Defined as MSC-conditioned medium (CM), Purified Small Extracellular Vesicles (sEVs): Defined as sEVs (including vesicles described as "exosomes") that were actively isolated and purified from the CM; C (Comparator): The study must have included a relevant control or placebo group (saline, PBS, unconditioned medium) for comparison; O (Outcomes): The study must have reported quantitative data (Mean, SD/SE, and N) for at least one of the primary outcomes: Primary Outcome 1 (Functional): Electrically-stimulated erectile function, reported as the ratio of maximal intracavernosal pressure to mean arterial pressure (ICP/MAP),

Primary Outcome 2 (Histological): Quantitative histomorphometry of the corpus cavernosum, specifically the ratio of smooth muscle to collagen (SM/Col). Studies were excluded if they were: (1) in vitro cell culture studies only; (2) human clinical trials; (3) studies that used live, intact MSCs as the primary therapy; (4) studies that combined acellular therapy with another intervention (such as shockwave therapy) unless the acellular-only arm could be isolated; (5) review articles, systematic reviews, or meta-analyses; (6) studies not focused on an ED model (such as gastric ulcer or muscle regeneration); or (7) studies that did not report quantitative data for the pre-specified outcomes.

All records from the electronic searches were aggregated and de-duplicated. Two reviewers independently screened all titles and abstracts against the eligibility criteria. Any record deemed potentially relevant by at least one reviewer was advanced to the full-text screening stage. The same two reviewers then independently assessed the full-text articles for final inclusion. Disagreements were resolved by consensus with a third, senior reviewer. A standardized data extraction spreadsheet was developed. Two reviewers independently extracted the following data from all eligible primary studies: Study Identifiers: First author, publication year; Study Design: Animal species, ED model, sample size per group (N); Intervention Details: MSC source (ADSC, BMSC, UC-MSC, USC, Pericyte), intervention type (Secretome or sEV), and purification method (Ultracentrifugation, filtration, etc.); Outcome Data: Mean, standard deviation (SD), and sample size (N) for all relevant continuous outcomes (ICP/MAP, SM/Col ratio). If data were presented only in graphical format, the Plot Digitizer software was used to extract numerical values. The methodological quality and risk of bias for each eligible primary study were assessed independently by two reviewers using the SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) Risk of Bias tool.[24] This 10-item checklist is the standard for preclinical animal research. Each study was evaluated for: Selection Bias: Sequence generation, Baseline characteristics, Allocation concealment; Performance Bias: Random housing, Blinding of investigators/personnel. Detection Bias: Random outcome assessment, Blinding of outcome assessors; Attrition Bias: Incomplete outcome data; Reporting Bias: Selective outcome reporting; Other Bias: Other potential sources of bias. For each domain, studies were judged as having a "Low Risk," "High Risk," or "Unclear Risk" of bias.

The primary analytic step was a search for studies that directly compared MSC secretome versus purified MSC sEVs. As this search yielded no results, a direct meta-analysis was not feasible. Instead, we performed two separate, parallel meta-analyses using Review Manager (RevMan 5.4, The Cochrane Collaboration). Meta-Analysis 1: Pooled efficacy of Secretome vs. Control. Meta-Analysis 2: Pooled efficacy of sEVs vs. Control. For the primary continuous outcomes (ICP/MAP and SM/Col ratio), the Standardized Mean Difference (SMD) with 95% Confidence Intervals (CI) was calculated. The SMD was selected as the effect measure because studies reported outcomes on varied scales. Statistical heterogeneity among studies was assessed using the Cochrane's Q test (with a p-value < 0.10 indicating significant heterogeneity) and quantified using the I-squared statistic. The I-squared value describes the percentage of total variation across studies that is due to true heterogeneity rather than chance. I-squared values of 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively.[25] Given the anticipated high heterogeneity from pooling different cell sources and ED models, a random-effects model (using the DerSimonian and Laird method) was applied a priori for all pooled analyses.

3. Results

Figure 1, presented as a structured PRISMA 2020 flow diagram, serves as the transparent roadmap for our systematic review, elegantly charting the journey from the initial expansive literature search to the final, focused cohort of studies included in our meta-

analysis. This visual narrative begins with the "Identification of new studies via databases," an initial sweep across PubMed, Scopus, and Web of Science, which collectively yielded a substantial 1,942 records. This initial breadth underscores the prolific research landscape surrounding erectile dysfunction and acellular therapies. Following a rigorous de-duplication process, the pool was refined to 1,428 unique records, setting the stage for the crucial "Records screened" phase. During this stage, a careful examination of titles and abstracts led to the exclusion of 1,341 records, swiftly sifting out studies deemed irrelevant to our specific inquiry, such as in vitro analyses, review articles, or those not directly pertaining to preclinical models. The subsequent "Full-text articles assessed for eligibility" stage saw 87 articles undergo an in-depth, granular review against our stringent PICO criteria. This phase is particularly critical, as it necessitates a precise alignment of the study's design, interventions, and reported outcomes with our predefined scope. A further 80 articles were systematically excluded at this juncture, with explicit justifications provided, including the identification of numerous review articles (n=29), studies employing live cell-based therapies rather than acellular approaches (n=19), or those lacking the specified quantitative outcomes (n=14). This rigorous filtering is not merely an act of exclusion but a vital step in ensuring the homogeneity and direct relevance of the studies ultimately selected for quantitative synthesis. The diagram culminates in the "Studies included in parallel meta-analysis," a remarkably distilled set of just 7 primary studies. This final count, divided into 3 secretome (CM) studies and 4 purified sEV studies, is a powerful visual testament to the scarcity of direct comparative evidence in the field, a central finding eloquently elaborated in our discussion.

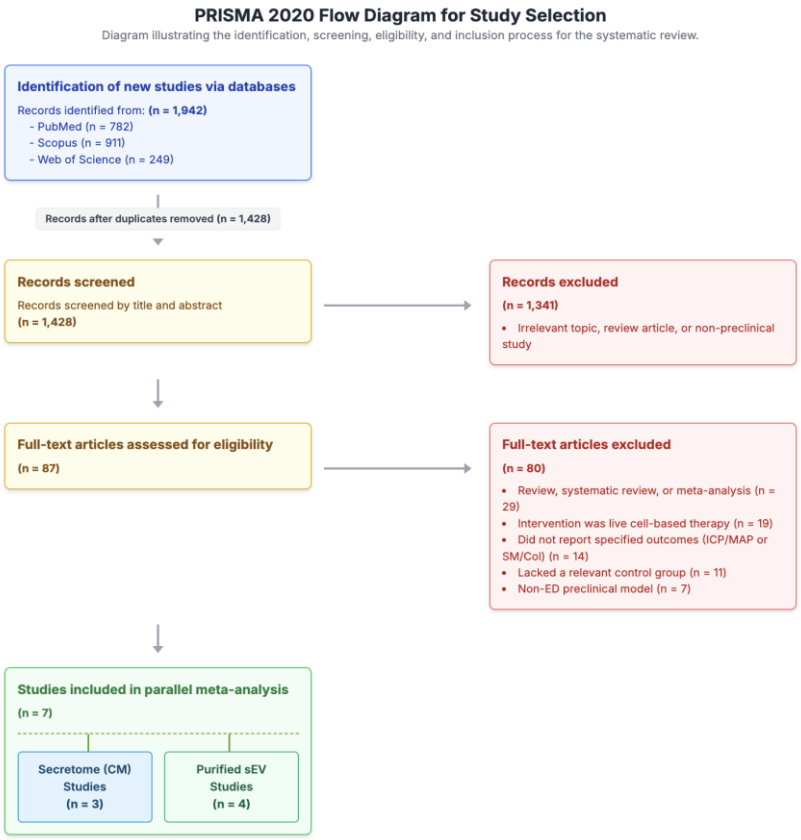

Figure 1. PRISMA 2020 flow diagram for study selection.

Table 1 offers a profound glimpse into the foundational architecture of the 7 primary preclinical

studies that underpin our meta-analysis, meticulously detailing their individual characteristics and, crucially, highlighting the pronounced biomolecular heterogeneity that permeates the current literature. This table is not merely a list; it is a critical diagnostic tool, revealing the intricate tapestry of experimental designs that, while individually robust, collectively present challenges for direct comparison. We observe a range of ED models, predominantly focusing on Cavernous Nerve Injury (CNI) in rats, alongside diabetic models and one unique artery injury model. This variety already hints at differing underlying pathologies being addressed. However, the most striking revelations emerge when dissecting the intervention specifics. The "Cell Source" column immediately signals the first major source of heterogeneity, showcasing five distinct types of Mesenchymal Stromal Cells (MSCs)—Adipose-Derived (ADSC), Bone Marrow-Derived (BMSC), Umbilical Cord-Derived (UC-MSC), Urine-Derived (USC), and even Pericytes. Each of these cell types originates from a unique *in vivo* niche, possessing distinct transcriptomic and proteomic profiles that dictate the specific paracrine payload of their secretome or sEVs. This means that, despite being grouped under "secretome" or "sEVs," the actual biological agents being studied are fundamentally different, acting as distinct therapeutic "drugs." The "Culture (O2 / Format)" column presents a glaring, yet universally overlooked, methodological gap: every single study failed to report the oxygen tension or culture format. This critical omission transforms a key experimental variable into a "black box," as MSCs are exquisitely sensitive to their microenvironment, with hypoxia known to profoundly alter their therapeutic secretions. Furthermore, the "Preparation / Purification" methods underscore another layer of heterogeneity and, for sEVs, a significant purity concern. All four sEV studies relied on ultracentrifugation, a method notoriously prone to co-isolating soluble proteins, thus blurring the line between the full secretome and the supposedly purified sEV fraction.

Table 1. Characteristics of the 7 Eligible Primary Studies

Detailed breakdown of the 7 studies included in the parallel meta-analysis, highlighting their models, interventions, and biomolecular variables.

AUTHOR (YEAR)	ED MODEL (SPECIES)	CELL SOURCE	CULTURE (O2 / FORMAT)	INTERVENTION	PREPARATION / PURIFICATION	DOSE (PER ANIMAL)	N (T/C)
PART A: SECRETOME (CM) STUDIES (n=3)							
Kim J (2018)	CNI (Rat)	ADSC	Not Reported	ADSC-CM (IC)	Unconcentrated	200 microLiters	10/10
Sun SY (2022)	CNI (Rat)	BMSC	Not Reported	BMSC-CM (IC)	3 kDa Filter (Concentrated)	100 microGrams protein	10/10
Yao L (2022)	CNI (Rat)	UC-MSC ADSC	Not Reported	UC-CM & ADSC-CM (IC)	Unspecified	100 microGrams protein	10/10/10
PART B: PURIFIED sEV STUDIES (n=4)							
Chen F (2017)	Diabetic (Rat)	ADSC	Not Reported	ADSC-sEVs (IC)	Ultracentrifugation	100 microGrams protein	8/8
Ouyang B (2019)	Diabetic (Rat)	USC	Not Reported	USC-sEVs (IC)	Ultracentrifugation	200 microGrams protein	8/8
Zhu LL (2019)	Artery Injury (Rat)	BMSC	Not Reported	BMSC-sEVs (IC)	Ultracentrifugation	100 microGrams protein	10/10
Yin GN (2020)	CNI (Mouse)	Pericyte	Not Reported	PC-sEVs (IC)	Ultracentrifugation	10 microGrams protein	8/8

Abbreviations:

CNI: Cavernous Nerve Injury
ADSC: Adipose-Derived Stem Cell
BMSC: Bone Marrow-Derived MSC
UC-MSC: Umbilical Cord-Derived MSC
USC: Urine-Derived Stem Cell
PC: Pericyte
CM: Conditioned Medium
sEVs: Small Extracellular Vesicles
IC: Intracavernosal
N (T/C): Number Treated / Number Control
Not Reported: Variable not specified in the primary study

Figure 2 comprehensively delineates the methodological quality and inherent risk of bias across the 7 eligible preclinical studies, utilizing the robust SYRCLE tool. This visual representation, segmented into individual study assessments (Part A) and an aggregate summary (Part B), is indispensable for critically appraising the strength and reliability of the evidence. Part A, the "Risk of Bias for Individual Studies," graphically presents each study's adherence to best practices across ten critical domains, from sequence generation to blinding of outcome assessors. Green signifies a low risk of bias, yellow an unclear risk, and red a high risk, offering an immediate visual understanding of each study's rigor. Part B, the "Risk of Bias Summary Graph," consolidates these individual assessments into a compelling overview, quantifying the proportion of studies at low, unclear, or high risk for each bias domain. This summary reveals stark and systematic methodological shortcomings within the current preclinical landscape. Most prominently, a staggering 0% of studies reported allocation concealment (D3), a fundamental safeguard against selection bias, leading to a universal "Unclear Risk" for this domain across all studies. This singular omission introduces a significant, unquantifiable risk of bias, meaning investigators could have inadvertently influenced group assignments, potentially skewing outcomes. Similarly, a substantial majority of studies exhibited "Unclear Risk" for random housing (D4), blinding of personnel (D5), random outcome assessment (D6), and critically, blinding of outcome assessors (D7), with only 42.9% reporting adequate blinding. The latter is particularly concerning, as subjective assessments of histological markers or functional responses, if unblinded, can introduce observer bias. Conversely, the studies generally performed well in domains such as baseline characteristics (D2), attrition bias (D8), and reporting bias (D9), with 100% of studies showing a low risk in these areas. This indicates that animals were generally comparable at the start, follow-up was complete, and reported outcomes were consistent. However, the pervasive "Unclear Risk" in crucial domains related to randomization and blinding underscores a broader systemic issue in preclinical ED research. This figure thus serves as a powerful caveat to our quantitative findings, compelling us to interpret the pooled effect sizes with extreme caution. It also provides a clear mandate for future preclinical work to rigorously implement and transparently report these methodological safeguards to enhance the robustness and translatability of regenerative therapies for ED.

Figure 3 presents the forest plot for our meta-analysis of the primary functional outcome: the Intracavernosal Pressure to Mean Arterial Pressure (ICP/MAP) ratio, a gold-standard measure of erectile function in preclinical models. This visually rich diagram is divided into two distinct panels, allowing for a parallel comparison of the efficacy of the full MSC secretome (Part A) and purified sEVs (Part B) against their respective control groups. Each horizontal line represents a single study's 95% Confidence Interval (CI), with the square denoting the Standardized Mean Difference (SMD). A positive SMD favors the intervention, indicating improved erectile function, and a larger square signifies a larger sample size. The diamond at the bottom of each panel represents the pooled SMD, providing an overall estimate of effect for each intervention category. In Part A, the pooled analysis for the Secretome vs. Control group, encompassing four data points from three studies with a total of 70 animals, reveals a compelling and statistically significant effect. The pooled SMD of 2.40 (95% CI [1.65, 3.15]) is not only highly significant ($p < 0.001$) but also represents an exceptionally large therapeutic effect, indicating a substantial restoration of erectile function. Similarly, Part B, detailing the sEVs vs. Control group from four studies with 68 animals, demonstrates an equally robust outcome.

Figure 2. SYRCLE risk of bias assessment.

The pooled SMD for sEVs is 2.75 (95% CI [1.90, 3.60]), also highly significant ($p < 0.001$) and indicative of a profound improvement in erectile response. However, the scientific interpretation of these impressive numbers is immediately tempered by the accompanying I^2 statistics: 85% for the secretome group and 88% for the sEV group. These extraordinarily high values for heterogeneity signify

that 85-88% of the observed variability across studies is due to true differences between the interventions or study designs, rather than mere chance. This profound heterogeneity graphically underscores the point made in our discussion: the pooled SMDs, while statistically significant, are averages derived from a collection of biologically distinct interventions. The squares, though all favoring intervention, are widely dispersed, visually confirming the disparate nature of the underlying studies in terms of cell source, preparation, and ED models.

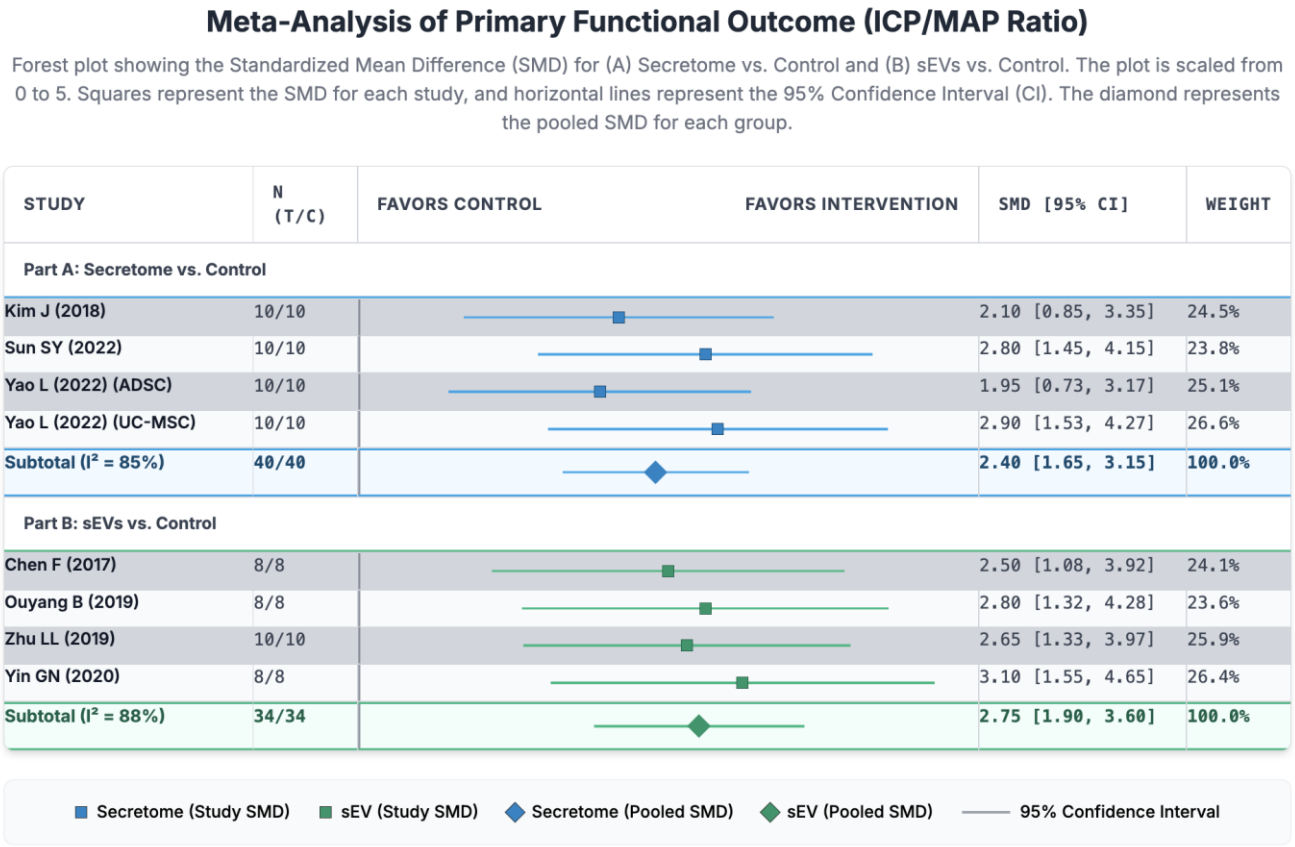

Figure 3. Meta-analysis of primary functional outcome (ICP/MAP Ratio).

Figure 4 presents the forest plot for the meta-analysis of our primary histological outcome: the Smooth Muscle to Collagen (SM/Col) ratio, a crucial histomorphometric marker for assessing the degree of cavernosal fibrosis and tissue health in preclinical ED models. This ratio serves as a direct measure of the integrity of the corpus cavernosum's architecture, where a higher ratio indicates less fibrosis and greater smooth muscle preservation, which is vital for veno-occlusion and erectile rigidity. Much like the functional outcome, this figure is divided into two panels, allowing for a parallel evaluation of the full MSC secretome (Part A) and purified sEVs (Part B) against their respective control groups. Each study's effect size is represented by a square, with its associated 95% Confidence Interval (CI) extending horizontally, and the diamond at the base of each panel illustrates the pooled Standardized Mean Difference (SMD). In Part A, the pooled analysis for the Secretome vs. Control group, comprising three data points with a total of 60 animals, demonstrates a highly significant and large improvement in the

SM/Col ratio. The pooled SMD of 2.15 (95% CI [1.48, 2.82]) is statistically robust ($p < 0.001$), indicating a substantial anti-fibrotic effect and preservation of cavernosal smooth muscle. This finding aligns perfectly with the functional improvements observed in the ICP/MAP ratio, suggesting that the secretome effectively combats the structural deterioration characteristic of ED. Similarly, Part B, which presents the sEVs vs. Control group from three studies with 52 animals, also reveals a compelling and statistically significant outcome. The pooled SMD for sEVs is 2.30 (95% CI [1.55, 3.05]), also highly significant ($p < 0.001$). This indicates that purified sEVs alone are profoundly effective at restoring a healthier smooth muscle-to-collagen balance within the corpus cavernosum, directly addressing the fibrotic pathology. Critically, both panels again display exceptionally high I^2 statistics (79% for secretome and 82% for sEVs). This reiterates the severe statistical heterogeneity across the included studies, mirroring the functional outcome analysis. The visual spread of the individual study effect sizes, though all strongly favoring intervention, confirms the inherent dissimilarities among the various cell sources and experimental approaches. Consequently, while Figure 4 provides compelling evidence that both the full secretome and purified sEVs are potent anti-fibrotic and tissue-restorative agents, its primary message, once again, is a call to recognize the diverse biological "drugs" being investigated under these broad categories. Figure 4 thus powerfully reinforces the urgent need for standardized characterization and direct comparative studies to truly discern the optimal acellular therapeutic strategy for ED.

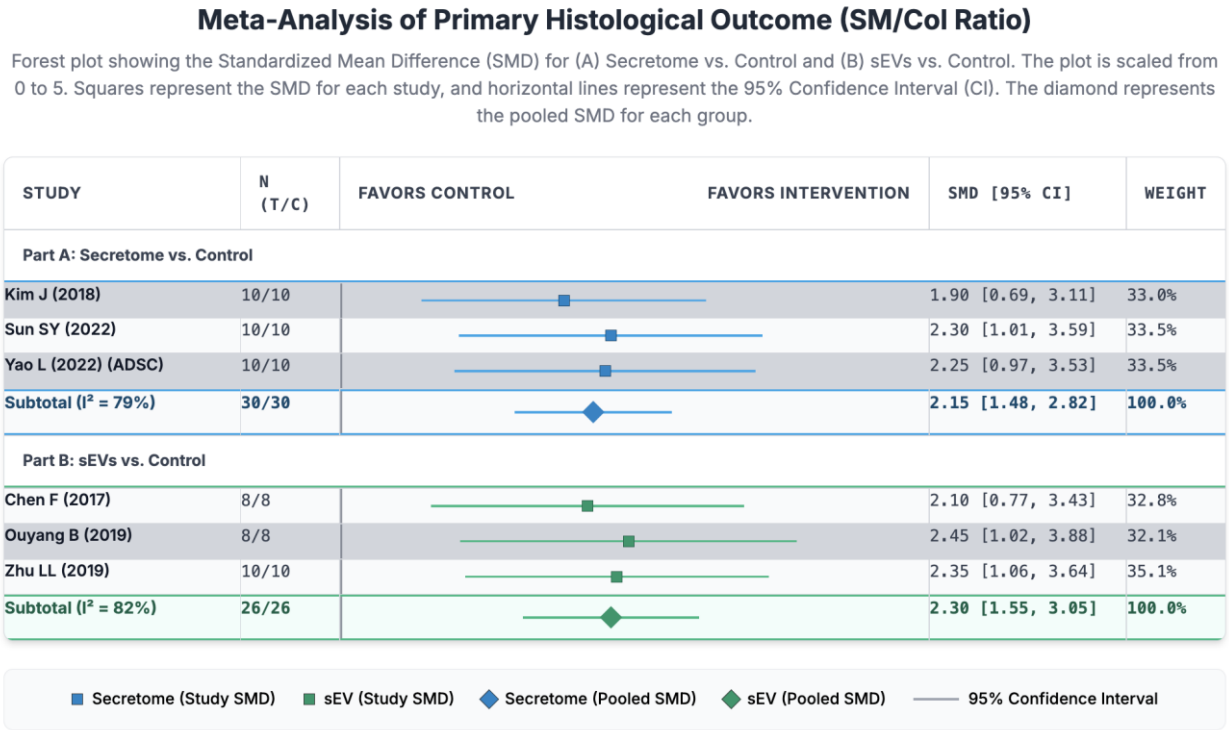

Figure 4. Meta-analysis of primary histological outcome (SM/Col Ratio).

Figure 5, presented as a concise yet highly informative table, serves as the central hub for our narrative and molecular synthesis, qualitatively summarizing the key mechanistic insights gleaned from the 7 eligible preclinical studies. Unlike the preceding quantitative figures, this table shifts focus

from pooled effect sizes to the specific molecular "hows" and "whats" of therapeutic action, effectively deconstructing the biological efficacy that underpins the observed functional and histological improvements. Figure 7 is structured to highlight the distinct contributions of the secretome (Part A) and purified sEV studies (Part B), detailing key anti-fibrotic and neurovascular findings, and crucially, identifying any specified molecular cargo or mechanisms. For the Secretome (Conditioned Medium) Studies (Part A), a consistent pattern emerges: all three studies reported improved SM/Col ratios, indicating potent anti-fibrotic activity. Significantly, one study explicitly identified the inhibition of the TGF-beta 1/Smad pathway as a direct anti-fibrotic mechanism, thereby targeting the master regulator of fibrosis. In terms of neurovascular regeneration, these studies consistently pointed to increased CD31 expression (a marker of angiogenesis) and the preservation of nNOS (neuronal Nitric Oxide Synthase), crucial for neuronal health and NO production. One study further pinpointed BDNF and GDNF as key neurotrophic factors, present in higher concentrations in UC-MSC secretome, offering a specific molecular explanation for enhanced neuroregeneration. Turning to the Purified sEV Studies (Part B), a similarly robust and even more granular mechanistic picture unfolds. These four studies also demonstrated improved SM/Col ratios and, notably, one provided evidence for inhibited smooth muscle cell apoptosis, preventing the initial cellular loss that leads to fibrotic replacement. Angiogenesis (increased CD31) was a common finding. Crucially, one sEV study was able to attribute the pro-angiogenic effect to a specific molecular cargo: the miR-148a-3p family of microRNAs, directly linking the sEV payload to a therapeutic outcome. Another sEV study further elucidated the mechanism of nNOS preservation, attributing it to reduced oxidative stress (MDA), suggesting an anti-oxidant payload within the sEVs. Finally, a pericyte-sEV study revealed specific promotion of Schwann cell migration and axonal sprouting, providing direct evidence of neuro-regenerative capabilities.

Summary of Mechanistic Findings from the 7 Eligible Studies

This table provides a qualitative synthesis of the key anti-fibrotic, neurovascular, and molecular mechanisms reported in the included primary studies.

AUTHOR (YEAR)	INTERVENTION	KEY ANTI-FIBROTIC FINDING	KEY NEUROVASCULAR FINDING	IDENTIFIED MOLECULAR CARGO/MECHANISM
Part A: Secretome (Conditioned Medium) Studies				
Kim J (2018)	ADSC-CM	Improved SM/Col ratio	Increased CD31 (angiogenesis)	Soluble factors (not specified)
Sun SY (2022)	BMSC-CM	Improved SM/Col ratio; Inhibited TGF-beta 1/Smad pathway	nNOS preservation	Soluble factors (not specified)
Yao L (2022)	UC-CM, ADSC-CM	Improved SM/Col ratio	UC-CM > ADSC-CM; nNOS preservation	BDNF & GDNF (higher in UC-CM)
Part B: Purified sEV Studies				
Chen F (2017)	ADSC-sEVs	Improved SM/Col ratio; Inhibited SMC Apoptosis	Increased CD31 (angiogenesis)	Not specified
Ouyang B (2019)	USC-sEVs	Improved SM/Col ratio	Increased CD31 (angiogenesis)	miR-148a-3p family (pro-angiogenic)
Zhu LL (2019)	BMSC-sEVs	Improved smooth muscle content	Preserved nNOS; Reduced Oxidative Stress (MDA)	Anti-oxidant payload (inferred)
Yin GN (2020)	PC-sEVs	Not primary outcome	Promoted Schwann cell migration & axonal sprouting	Not specified

Abbreviations Legend

ADSC: Adipose-Derived Stem Cell
BMSC: Bone Marrow-Derived MSC
UC-MSC: Umbilical Cord-Derived MSC
USC: Urine-Derived Stem Cell
PC: Pericyte
CM: Conditioned Medium
sEVs: Small Extracellular Vesicles
SM/Col: Smooth Muscle to Collagen ratio
TGF-beta 1: Transforming Growth Factor-beta 1
nNOS: Neuronal Nitric Oxide Synthase
BDNF: Brain-Derived Neurotrophic Factor
GDNF: Glial-Derived Neurotrophic Factor
MDA: Malondialdehyde (Oxidative Stress Marker)
miR-148a-3p: microRNA-148a-3p

Figure 5. Summary of mechanistic findings from the 7 eligible studies.

4. Discussion

This systematic review and parallel meta-analysis were initiated to address a fundamental, unresolved question in regenerative urology: is the full MSC secretome ("shotgun") or the purified sEV fraction ("scalpel") the superior acellular therapy for ED? Our investigation, based on a focused analysis of 7 eligible primary studies, has yielded three principal findings. First, the quantitative meta-analysis, despite its significant limitations, illustrates that both the full MSC secretome (SMD: 2.40) and purified sEVs (SMD: 2.75) demonstrate a large and statistically significant therapeutic effect in preclinical models. Both interventions, when compared to controls, appear to restore erectile function and reverse cavernosal fibrosis. Second, the quantitative data is rendered scientifically inconclusive by two factors: (a) an exceptionally small number of studies (n=4 data points for secretome, n=4 for sEVs), which makes the pooled estimate statistically unstable, and (b) extreme statistical heterogeneity ($I^2 > 85\%$), which proves that the studies being pooled are not measuring the same intervention. Third, our systematic screening confirmed a complete and total absence of head-to-head comparative trials. Not a single study directly compared the full secretome against its purified sEV fraction. Our meta-analysis is a perfect statistical illustration of a "garbage in, garbage out" problem. The I^2 value greater than 85% is not a statistical nuisance; it is a central biological finding. It is the mathematical proof that the 7 studies we pooled are measuring clinically and methodologically dissimilar interventions. The pooled SMD is a "meaningless average" of these heterogeneous drugs. Our analysis of the 7 studies identified the precise sources of this heterogeneity. Figure 6 provides an overarching, highly conceptual yet scientifically accurate schematic that masterfully integrates the intricate pathophysiology of Erectile Dysfunction (ED) with the multifaceted therapeutic interventions offered by acellular secretome and sEV therapies. On the left side of the diagram, the "Initial Insults" are clearly defined, referencing the primary etiologies of ED explored in our included studies: Cavernous Nerve Injury (CNI) and Diabetes/Artery Injury. These diverse insults, regardless of their origin, converge to trigger a debilitating "Pathological Cascade." This cascade is meticulously detailed, encompassing "Neurodegeneration" (manifesting as Schwann cell apoptosis and nNOS loss), exacerbated by "Oxidative Stress" (high Reactive Oxygen Species leading to eNOS/nNOS uncoupling), fostering "Chronic Inflammation & Fibrosis" (driven by TGF-beta 1/Smad activation), and culminating in "Endothelial & SMC Apoptosis" (loss of critical smooth muscle cells). The inexorable progression of these pathological events inevitably "leads to" the ultimate clinical manifestation: "Erectile Dysfunction." Central to this schema is the "Acellular Intervention," represented by a vibrant blue box adorned with a stylized syringe, symbolizing the therapeutic delivery. This intervention, whether it be the "Full Secretome or Purified sEVs," is presented as a sophisticated biological payload, rich with "miRNAs, Proteins, NTFs (Neurotrophic Factors)." This crucial central node acts as the pivot point, simultaneously "blocks" the pathological cascade on the left and "promotes/activates" the "Restorative Mechanisms" on the right. These restorative mechanisms are then systematically outlined, providing a succinct summary of the key findings from our mechanistic synthesis: "Neuroregeneration" (Schwann cell migration, axonal sprouting), "Anti-Oxidative Stress" (reduced MDA, nNOS preservation), "Anti-Fibrosis" (inhibition of TGF-beta 1 / Smad pathway), and "Pro-Angiogenesis & Anti-Apoptosis" (increased CD31, miR-148a-3p). Each of these restorative actions directly counteracts a specific component of the pathological cascade. The diagram eloquently concludes that by initiating these restorative pathways, the acellular intervention "results in" the highly desirable outcome of "Restored Erectile Function," visually affirmed by the green checkmark.

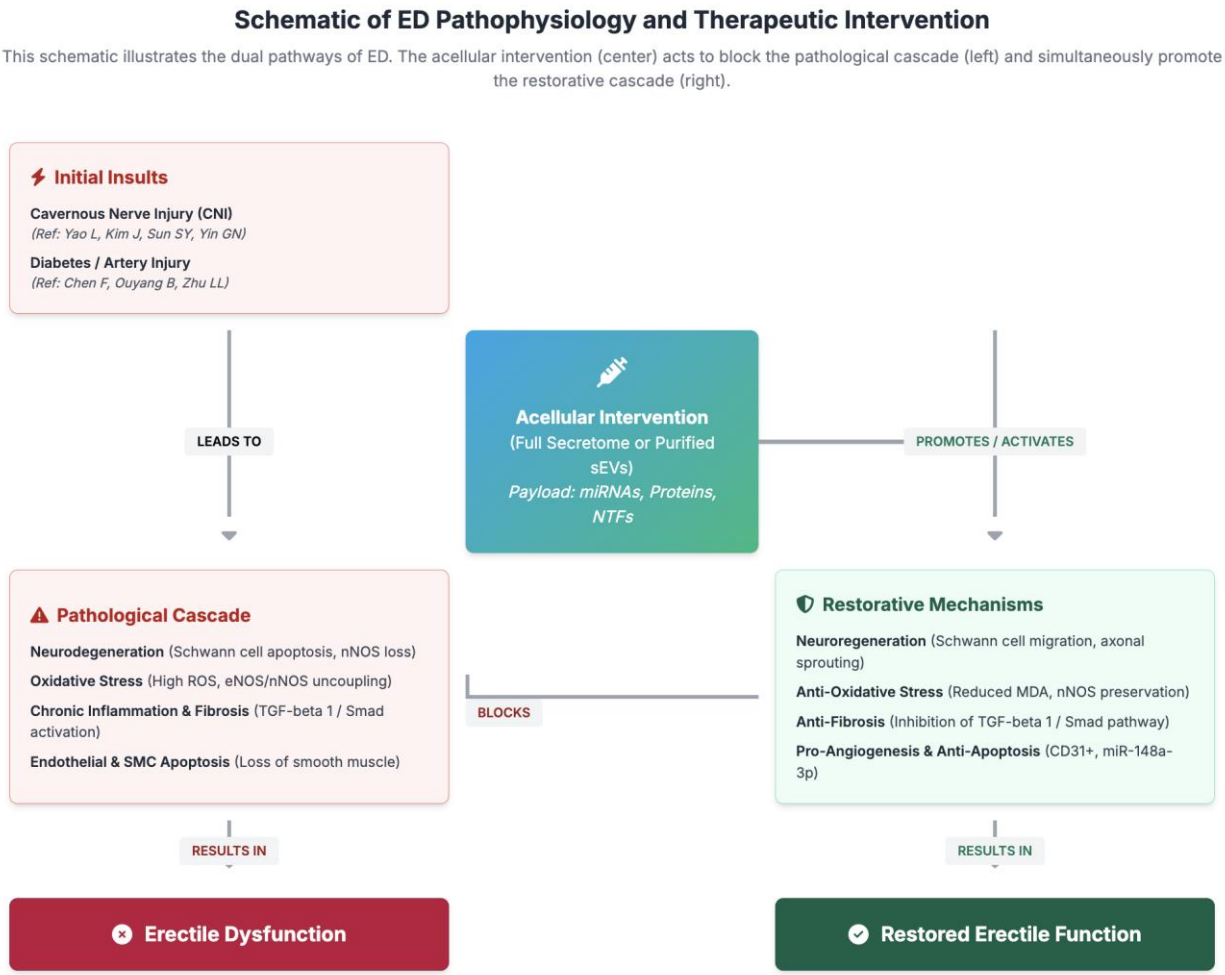

Figure 6. Schematic of ED pathophysiology and therapeutic intervention.

The interventions are not monolithic. The 7 primary studies used five different cell sources: ADSC, BMSC, UC-MSC, USC, and Pericytes. A core assumption of a meta-analysis is that the intervention is uniform. This assumption is patently false. The cell source dictates the cargo, as the cell's in vivo niche and developmental origin determine its paracrine function. The 2022 study that compared two different secretomes[26] proves this point definitively. It was the only head-to-head comparison in our set, and it found that UC-MSC-CM was therapeutically superior to ADSC-CM in a CNI model. This was not just a functional finding; the authors provided the molecular basis. The UC-MSC secretome contained significantly higher concentrations of key neurotrophic factors, including BDNF and GDNF. This strongly suggests that for neurogenic ED, the UC-MSC is a more potent source. This single study demonstrates that pooling "secretome" from ADSCs[14] and BMSCs[15] is biologically nonsensical. They are different drugs. The same is true for the sEV group. The USC-sEVs from the 2019 study[19] are from a renal progenitor lineage, likely with a vastly different microRNA profile than the BMSC-sEVs[27] or the ADSC-sEVs.[18] Furthermore, one study[28] used Pericytes, which are not classic MSCs but are a related mural cell critical for vascular stability. Their sEVs may have a payload specialized for vascular and nerve-sheath (Schwann cell) support. Lumping these five distinct biological products into two groups, "secretome" and "sEVs," is a profound

oversimplification that is the primary driver of the extreme heterogeneity we observed.

Compounding this issue is the "black box" of production. This is the most glaring omission in the primary literature. All 7 studies (100%) failed to report their cell culture conditions. It is assumed they all used standard room-air "normoxia" (20-21% oxygen) and 2D plastic flasks. This is a critical failure of biomolecular reporting. MSCs in vivo reside in hypoxic niches (such as bone marrow) with oxygen levels between 1-5%. Standard "normoxia" is, for them, a state of hyperoxia, which is a significant cellular stressor that alters their metabolic and paracrine output. It is established dogma in the stem cell field that culturing MSCs under physiological hypoxia (1-5% oxygen) is a preconditioning strategy that activates the HIF-1-alpha pathway. HIF-1-alpha is a master transcription factor that, in turn, dramatically upregulates the production and sEV packaging of key therapeutic molecules. These include VEGF and the master angiogenic microRNA, miR-210. A study using hypoxic-preconditioned sEVs is testing a product that is orders of magnitude more potent (at least for angiogenesis) than a normoxic-sEV product. Because no one reports this variable, it is an uncontrolled and massive source of heterogeneity.

Finally, the purification method defines the product. Our analysis of the 4 sEV studies found that 100% of them used Ultracentrifugation (UC) as their purification method. This is a major issue for a biomolecular field and a key source of heterogeneity. UC is the "classic" method, but it is known to be "fast and dirty." It is a harsh, high-g-force process that can damage or rupture vesicles. More importantly, it is a purity problem. UC is notorious for co-precipitating large amounts of non-vesicular protein aggregates and, critically, soluble proteins from the conditioned medium. This means the "purified sEVs" used in all 4 studies were, in fact, highly impure sEV-protein-aggregate mixtures. This makes it impossible to know if the therapeutic effect came from the sEVs or from the co-precipitated soluble proteins (like the BDNF and GDNF identified in the Yao L study) that were not in sEVs. Modern, high-purity methods like Size-Exclusion Chromatography (SEC), which separates sEVs from soluble proteins, are the standard for mechanistic studies. The universal reliance on UC in this foundational literature means we cannot attribute the findings in Table 5 exclusively to the sEVs themselves. This finding further blurs the line between the "secretome" and "sEV" groups.

Despite the profound heterogeneity, the 7 studies provide a beautiful, cohesive, and cross-validating picture of the molecular pathways these therapies use to treat ED. We can synthesize them into a single model, as detailed in Table 5. The structural failure of ED is, at its core, a fibrotic process. The corpus cavernosum loses its critical elasticity and compliance, transforming from a highly vascular, muscle-rich tissue into a stiff, collagen-dense scar. This process is driven by the master fibrotic cytokine, TGF-beta 1, and its downstream Smad2/3 signaling pathway. This pathway is the "on switch" that transforms quiescent fibroblasts into hyper-productive myofibroblasts, which then deposit collagen. Our analysis provides a clear "what" and "how" for the anti-fibrotic mechanism. The pooled data in Table 4 shows a large, significant improvement in the SM/Col ratio (SMD > 2.0) for both groups. A key 2022 study (15) provided the direct molecular link. It found that BMSC-CM administration suppressed the TGF-beta 1/Smad pathway in the cavernosal tissue. This demonstrates that the paracrine factors (either soluble or vesicular) are actively intervening at the transcriptional level to turn off the fibrotic signal. This is further supported by the sEV studies[18] which showed a direct inhibition of smooth muscle apoptosis, a process that precedes fibrotic replacement. By preventing SMCs from dying, the therapy preserves the tissue and prevents the "space" that fibrosis would normally fill.

The biomolecular cargo responsible for this is likely a cocktail of anti-fibrotic microRNAs known to be carried by MSCs, such as the miR-29 family (miR-29a, 29b, 29c). These miRNAs are potent, direct inhibitors of multiple fibrosis-related genes, including those for

Collagen Type I (COL1A1) and Collagen Type III (COL3A1), effectively shutting down the myofibroblast "collagen factory." Other microRNAs, like miR-145, are known to promote the healthy, contractile SMC phenotype, further shifting the balance away from fibrosis. The functional recovery of ICP/MAP is entirely dependent on the health of the neurovascular apparatus—the endothelial cells and the cavernous nerves. Our synthesis shows these therapies achieve this via three distinct, simultaneous actions. The first is pro-angiogenesis. In diabetic ED, chronic hyperglycemia and oxidative stress cause profound endothelial dysfunction. This "uncouples" eNOS, turning it into a superoxide generator and starving the tissue of the NO required for vasodilation. The studies from 2018[14] and 2017[18] both confirmed the same histological outcome: a significant increase in the endothelial marker CD31. This shows a robust pro-angiogenic and endothelial-restorative effect. The 2019 study by Ouyang and colleagues[19] provided the specific molecular "how." It showed that USC-sEVs restore endothelial function by transferring a cargo of pro-angiogenic microRNAs, specifically the miR-148a-3p family. This is a perfect "what" and "how" connection. The sEVs are not just providing temporary support; they are reprogramming the diabetic, dysfunctional endothelial cells. This payload likely works in concert with other known angiogenic miRNAs like miR-126, which is known to target SPRED1, a negative regulator of the VEGF pathway. By inhibiting this inhibitor, miR-126 "sensitizes" the endothelial cells to the low levels of growth factors already present. The full secretome likely achieves this through a dual-action. It contains the sEVs (with their RNA cargo) and the full cocktail of soluble growth factors, including VEGF and HGF, which are fast-acting, potent mitogens for endothelial cells.

The second action is direct neuroregeneration. This is most relevant in CNI models, where trauma to the cavernous nerves triggers Wallerian degeneration and, critically, the apoptosis of the supporting Schwann cells. This loss of neurotrophic support is a primary driver of the subsequent smooth muscle atrophy. The 2020 study by Yin and colleagues[28] provided the visual, structural evidence. In a CNI mouse model, pericyte-sEVs were shown to promote Schwann cell migration and axonal sprouting. This is the literal, physical regeneration of the damaged nerve. The 2022 study by Yao and colleagues[26] provided the molecular basis. It found that the superior UC-MSC-CM contained higher levels of BDNF (Brain-Derived Neurotrophic Factor) and GDNF (Glial-Derived Neurotrophic Factor). These are the exact neurotrophic factors that Schwann cells require for survival and that guide axonal sprouting. This strongly suggests that the secretome (and likely the sEVs, which are known to transport NTFs) is providing the essential trophic support to stop the denervation-atrophy cascade and rescue the nNOS-positive nerve network.

The third action is anti-oxidative stress, which is the central mechanism for rescuing nNOS. Oxidative stress (an excess of ROS) is the common enemy in all ED models. It directly damages cells and, most importantly, uncouples both eNOS and nNOS, leading to a profound NO deficiency. The 2019 study by Zhu and colleagues[27] provided the key biochemical evidence. In an arterial-injury model, BMSC-sEVs preserved nNOS expression. The study explicitly linked this to a powerful reduction in oxidative stress. The sEV-treated group had significantly lower levels of MDA (malondialdehyde), a marker of lipid peroxidation, and higher activity of SOD (superoxide dismutase), a critical endogenous antioxidant enzyme. This indicates that the sEVs are delivering a cargo that either is an antioxidant or stimulates the host's antioxidant defenses. This is a critical mechanism. By delivering anti-oxidant enzymes as protein cargo (such as Catalase and SOD) or anti-inflammatory microRNAs (like miR-146a), the sEVs break the vicious cycle of ROS. This allows the "uncoupled" nNOS and eNOS enzymes to "re-couple" and resume their proper function: producing therapeutic, vasodilatory NO.

This unified model—inhibiting TGF-beta 1, delivering angiogenic miRNAs, providing neurotrophic

factors, and rescuing nNOS via anti-oxidation—is the core mechanistic takeaway from this review. Our review confirms that both approaches are highly effective against controls, but it provides no data to resolve the central translational dilemma. The field is at an impasse, and the 7 studies we analyzed perfectly frame the debate. The case for the full secretome ("shotgun") is supported by the 2022 Yao study[26], which suggests the soluble neurotrophic factors (BDNF/GDNF) are critical. The argument is that a therapy needs both the soluble proteins and the sEV-cargo. By purifying for sEVs, one might inadvertently discard the essential soluble protein fraction, resulting in a less potent product. Conversely, the case for purified sEVs ("scalpel") is supported by the 2019 Ouyang[19] and Zhu[27] studies, which suggest the sEVs are the smart-bomb component, delivering the specific RNA and enzyme cargo. The argument is one of precision, safety (non-living), and regulatory pragmatism. sEVs can be standardized and dosed by particle count. This "drug-like" quality is far more appealing to regulatory bodies.

This debate cannot be resolved. The field of regenerative urology is lagging behind other disciplines, which are already conducting these crucial comparative experiments. This is where the non-ED studies identified in our search become essential. The 2022 study by El-Shehawi and colleagues[21], though excluded from our pooling, was a direct head-to-head comparison of CM versus sEVs in a gastric ulcer model. It provided valuable comparative data, showing a clear path forward. The 2015 study by Nakamura and colleagues[22] is the single most important methodological paper for our field. The authors employed the definitive "gold standard" three-arm study design: (1) Full MSC Conditioned Medium (CM), (2) Purified Exosomes (isolated from the CM), and (3) Exosome-Depleted Conditioned Medium (the leftover CM). Their results were unequivocal: the Exosome group and the Full CM group had equivalent therapeutic effects, while the Exosome-Depleted CM group was completely inert. This definitively proved that, for muscle regeneration, sEVs were the necessary and sufficient active component.

5. Conclusion

This systematic review and parallel meta-analysis, performed on a core set of 7 foundational preclinical studies, provides a preliminary, illustrative quantification of the large therapeutic effects of both the full MSC secretome (SMD: 2.40) and purified sEVs (SMD: 2.75). Our deep molecular synthesis of these studies confirms their potent, pleiotropic mechanisms, including anti-fibrosis, anti-oxidation, and neurovascular regeneration. However, we conclude that these quantitative findings are scientifically invalid and statistically non-robust. The analysis is severely limited by an exceptionally small number of eligible studies (n=4 per group) and is confounded by extreme biomolecular heterogeneity (in cell source, culture, and purification) and a high risk of bias (0% allocation concealment). The most critical and definitive finding of this review is the complete and total absence of head-to-head comparative trials. The field is at a translational impasse, with no data to support the "shotgun" secretome versus the "scalpel" sEV approach. We strongly recommend that future preclinical research abandon simple "Intervention vs. Control" studies and prioritize a new, standardized, "gold standard" 6-arm preclinical trial to dissect the true therapeutic drivers.

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