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Review Paper

The Therapeutic Effects of Mesenchymal Stem Cells Derived Extracellular Vesicles on Intrauterine Adhesions

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ABSTRACT

Intrauterine adhesions (IUAs), also known as Asherman syndrome, is a pathological condition characterized by the development of fibrous scar tissue within the uterine cavity, leading to menstrual abnormalities, infertility, and recurrent pregnancy loss. Current treatment options for IUAs are limited and often associated with suboptimal outcomes. In recent years, mesenchymal stem cells (MSCs) and their secreted extracellular vesicles (EVs) have emerged as potential therapeutic tools for various tissue injuries and disorders. MSCs play an important role in regeneration and repair and can differentiate into several lineages. These cells can be harvested from various sources, such as bone marrow, umbilical cord, adipose tissue, peripheral blood, and placenta. EVs are small membrane-bound vesicles containing a diverse cargo of proteins, lipids, and nucleic acids, which can be transferred to target cells to modulate their biological functions. Evidence suggests that EVs possess therapeutic properties similar to their parent cells but without the risks associated with cell-based therapies. Studies have demonstrated that EVs, by multiple pathways and mechanisms, can promote endometrial repair, reduce fibrosis, and restore normal uterine function in animal models of IUAs. Understanding the therapeutic effects of MSCs-derived EVs on IUAs could pave the way for developing novel and minimally invasive treatment options for this challenging condition. This review provides an overview of the current knowledge regarding the therapeutic potential of different sources of MSC-EVs in treating IUAs in preclinical and in vitro studies.

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Introduction

Intrauterine adhesions (IUAs), also known as Asherman syndrome, is a medical condition characterized by the formation of scar tissue within the uterus and or cervix [1, 2]. This condition is identified by the presence of adhesions that have been confirmed through hysteroscopy [3, 4]. IUAs result from injury, severe infection, or hypoxia of the endometrial lining of a pregnant uterus [5, 6]. Moreover, it elicits clinical manifestations, such as hypomenorrhea, amenorrhea, infertility, cyclic lower abdominal pain, or recurrent pregnancy loss [7, 8]. It is suggested that the development of IUAs is caused by fibrosis of the uterine walls following the destruction of the endometrial basalis layer. This pathological phenomenon is characterized by the unregulated extracellular matrix (ECM) and fibrillar collagens accumulation. The stromal compartment undergoes fibrous tissue replacement, while the glands undergo replacement by inactive cubo-columnar epithelium. Moreover, IUAs are distinguished by heightened levels of pro-fibrosis and pro-inflammatory cytokines, namely interleukin-6 (IL-6) and interferon gamma (IFN-γ) [9]. The occurrence of IUAs is closely associated with epithelial-mesenchymal transition (EMT), which is recognized as one of the most significant mechanisms of fibrotic diseases [10]. The process of EMT is a complex biological phenomenon that may entail the activation of one or multiple signaling pathways [11, 12] (Figure 1). Therefore, any potential treatment for IUAs should be able to target these pathologic mechanisms. At present, hysteroscopic surgery is considered the favored approach for the removal of adhesions, complemented by hormone therapy, particularly estrogen therapy, to promote the regeneration of the endometrium and mitigate the formation of adhesions [13]. Nevertheless, the treatment of severe and dense IUAs poses a significant therapeutic challenge and is associated with a generally unfavorable prognosis with a recurrence rate of up to 62.5% [14], and the pooled pregnancy rate from 22.5% to 33.3% [15]. With the increasing popularity of minimally invasive techniques, the IUA treatment has shifted from the traditional approach of hysterotomy to alternative methods such as hysteroscopic resection, intrauterine devices, hormones, adhesion barriers, and stem cells [16]. Stem cell therapy is a leading alternative for invasive therapies, primarily operating via paracrine mechanisms [17, 18].

Mesenchymal stem cells (MSCs)

The MSCs are stem cells that play an essential role in regeneration and repair due to their capacity to differentiate into multiple lineages. They possess the capacity to differentiate into connective tissues, skeletal muscle cells, and vascular cells [19]. MSCs can be collected from various sources, including bone marrow, umbilical cord, adipose tissue, peripheral blood, and so on [20]. Significant potentials of proliferation, differentiation, and immunological regulation make MSCs a popular choice for use in cell therapy today [21]. New research suggests that MSCs have an immunomodulatory and homeostatic function, and their use may provide a means of controlling inflammation and speeding the healing of injured tissue in inflammatory conditions [22]. Furthermore, MSCs are considered a potential treatment option for IUAs [23, 24]. MSCs have demonstrated the ability to enhance endometrial function by releasing anti-inflammatory cytokines and growth factors, either directly [25, 26] or via extracellular vesicles (EVs) [27, 18, 28], resulting in tissue repair. Nevertheless, MSC treatment does have certain limitations. The transplantation of allogeneic MSCs into the body has the potential to mount an immune response leading to rejection [29]. In addition, stem cell therapy may give rise to concerns regarding transportation, storage, commercialization, rejection pathways, and safety issues related to inadequate monitoring tests [30-32]. One approach to address these issues is to employ autologous MSCs as a ready-made product. In addition, novel products such as acellular exosomes and MSCs generated from human pluripotent stem cells are gaining much attention [33].

EVs

EVs are a type of double membrane-enclosed vesicles that are shed by cells and are of nano-size [34, 35]. Unlike MSCs, there is substantial evidence supporting the involvement of EVs as novel communication paradigms that facilitate the transmission of biological information between cells in the field of regenerative medicine [36-38]. EVs are released by all types of cells in both normal and abnormal conditions [39]. They transport many large molecules, such as proteins, nucleic acids, and lipids [40]. EVs can enter target cells and deliver their cargo within these cells. Alternatively, they can bind to specific molecules on the surface of target cells and initiate internal signaling pathways [41, 42]. The term “EV” is a broad term used to refer to several types of vesicles that are released by cells, such as exosomes, apoptotic bodies, or microvesicles [43]. Every cell releases various types of EVs that vary in terms of their contents. As a re-

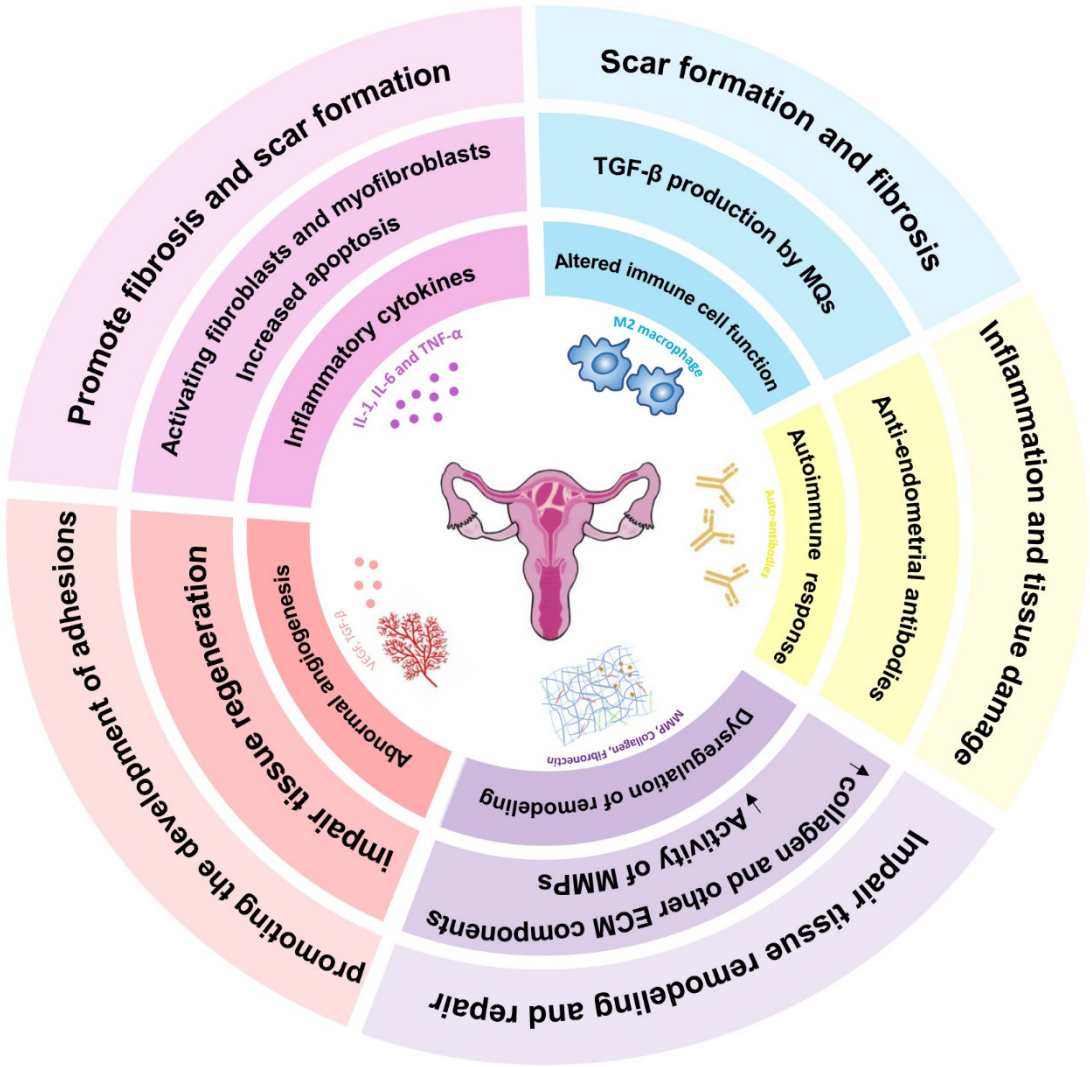

Figure 1. Different aspects of IUAs pathogenesis

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sult, these EVs may exhibit variances in physicochemical features, including density and size [44]. EVs membranes are identified by specific markers (CD63, CD9, CD81), and within them are cytoplasmic inclusions such as micro-ribonucleic acid (RNA), peptide hormones, and cytoplasmic and organelle-specific proteins. Unlike micro-vesicles, which are generated by the protrusion of a piece of the plasma membrane, exosome biogenesis occurs in multivesicular bodies or late endosomes [45]. Homing of MSC-derived EVs (MSC-EVs) to inflamed or damaged tissues is regulated by adhesion molecules like CD44, CD29, and CD73 [46]. When MSC-EVs reach their target cells, they may activate signaling via receptor-ligand contact or be absorbed by endocytosis to deliver their payload [47, 48]. The paracrine effects of MSC-EVs are primarily responsible for the immunomodulatory therapeutic benefits of MSC treatment that have been mentioned earlier [49, 50]. MSCs-EVs have been extensively utilized for tissue healing in both animal models and clinical trials [51]. Different from MSCs, EVs do not proliferate, making them easier to store and transfer. Additionally, the dosage of EVs is not restricted by issues like microvascular plugging or loss of viability, and they are highly immune-tolerant [52, 53]. The exceptional qualities of EVs make them suitable for immediate usage and allow for thorough and repeated testing before being used in healthcare settings. Before their implementation in the clinical environment, EVs must undergo strict quality control measures, regardless of their intriguing and efficient therapeutic potential [54, 55] (Figure 2).

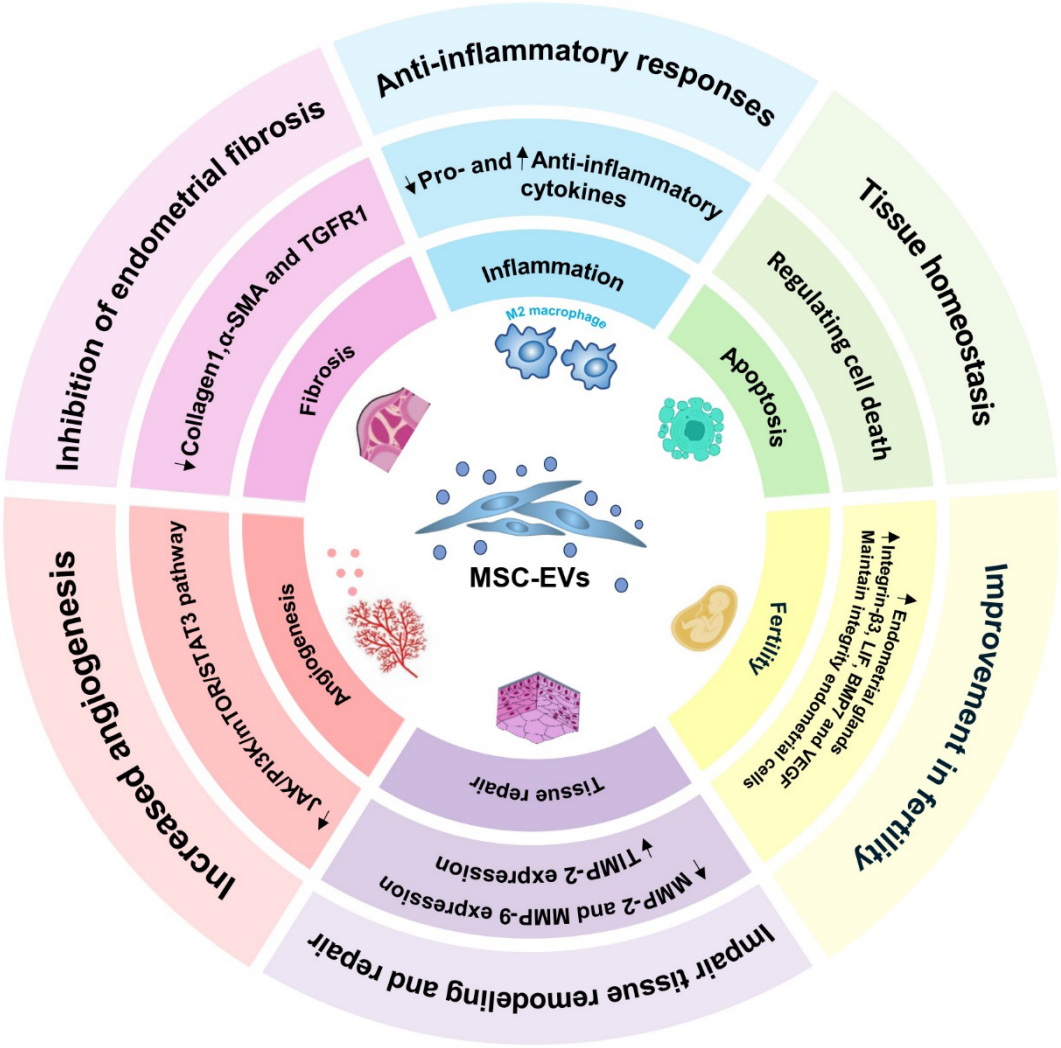

Figure 2. Schematic illustration of the effect of EVs derived from MSCs on tissue remodeling and repair IMMUNOREGULATION

Isolation and characterization of MSCs-EVs

There has been a rapid surge in EV research, with numerous methods available for separating them based on size, density, and immunoaffinity. However, ultracentrifugation remains the most often-used approach [56]. This technique utilizes a step-by-step process of centrifugation to separate cells and debris, followed by high-speed centrifugation at 100000-200000×g to isolate EVs [57]. Additional EV purification techniques enhance the purity of target EVs while reducing their amount. Size exclusion chromatography, ultrafiltration, immuno-based capturing, and chemical precipitation methods are more rapid and efficient in isolating EVs compared to ultracentrifugation [58]. The practice of using multiple isolation strategies to enhance the efficiency of EV isolation is increasingly prevalent. The combination of ultrafiltration and size exclusion chromatography effectively isolates EVs with a high yield while also maintaining their functional properties [56, 59]. Efficient methods are required to measure the size and concentration of EVs in biological fluids for medical and pharmacological applications. The predominant technique employed is nanoparticle tracking analysis, which utilizes a light microscope to monitor changes in vesicle light scattering caused by Brownian motion [60, 61]. Dynamic light scattering and nanoparticle tracking analysis both rely on the detection of nanoparticles (NVs) through their Brownian motion. However, dynamic light scattering was found to be imprecise when analyzing samples with varying particle sizes, as the detection element collected scattered light from all particles simultaneously [62]. The scanning electron microscope (SEM) and transmission

electron microscope (TEM) are often used to visualize the form and structure of EV [63]. Sample preparation can have an impact on the morphology and integrity of EVs when using SEM/TEM. To overcome barriers, cryo-electron microscopy (cryo-EM) is employed for EV investigation under the conditions of liquid nitrogen [64]. Atomic force microscopy is capable of directly measuring samples in their natural form, requiring no sample preparation, and generating a high-resolution 3D image of the surface topography [65]. Over the years, protein-based and RNA-based EV molecular profiling techniques have developed to offer a more extensive range of information. The quantification of nucleic acids can be achieved using several methods such as spectrophotometers, RiboGreen assay, quantitative reverse transcription polymerase chain reaction (qRT-PCR), next-generation sequencing (NGS)/RNA-seq, NanoString, and microarrays [66]. Western blot and enzyme-linked immunosorbent assay (ELISA) are protein detection technologies that rely on immunoaffinity. Potential methods for identifying EV proteins include reagent-based bicinchoninic acid and Bradford (Coomassie dye) tests, fluorescent reagent-based fluorometric assays, microfluidic approaches, and electrochemical detection [65, 67, 68].

Animal and in vitro models of IUAs

To construct an in vitro model of IUAs, several studies added TGF-ß [69, 70] or mifepristone [71] to ESCs to mimic the endometrium damage. Subsequently, following the addition of EVs to the culture medium, the expression of fibrotic markers was evaluated. In vivo, the modeling approaches for IUA can be classified into three main categories: Physical injury, chemical injury, and dual injury [72]. Physical injury methods include heat injury [73], electric scraping [74] and mechanical damage using a curette or blade [75, 76] in nonpregnant rats. Chemical injury approaches include the use of substances such as ethanol [77, 78], phenol mucilage [79], or trichloroacetic acid [28]. Dual injury methods refer to the simultaneous occurrence of two or three damage procedures to establish an IUA model. These approaches primarily require uterine curettage combined with lipopolysaccharide (LPS) treatment [80]. Once the animal model is established, different doses of EVs can be administered to the animal through several routes, such as IU [81], intraperitoneal (IP) [28], or intravenous (IV) [82] administration pathways. After treatment, the uterus of animals should be harvested, fixed, and stained to be investigated. Thickness, fibrotic area, and the number of glands are among the most important factors that should be measured in the uterus. In addition, several studies reported pregnancy outcomes after mating the animal models with males, which is a valuable result. The assessment of fertility serves as the gold standard for evaluating the efficacy of interventions in infertility management, providing a more direct reflection of therapeutic effects [27]. In the following, this study reviews the effects of MSC-EVs from multiple sources on the cell or animal models of IUAs (Table 1).

MSCs derived EVs

Bone marrow-derived EVs

Bone marrow MSCs (BMSCs) are a specific form of adult stem cells that can differentiate into many cell types. BMSC is a highly suitable source of stem cells in tissue engineering [91]. BMSCs are currently utilized in IUA treatment [92, 93]. While BMSCs do have a specific therapeutic impact on IUAs, they are also associated with challenges such as immune rejection, cell stability, and ethical concerns. Furthermore, the precise mechanism by which transplanted BMSCs facilitate endometrial regeneration is not well understood. Hence, it is imperative to discover an alternative approach to address the challenges encountered in the therapeutic management of stem cells [83]. Exosomes derived from BMSCs (BMSCs-Exos) can enhance cell proliferation and cell migration in vitro. Additionally, these EVs can effectively restore damaged endometrium in a mouse model of IUAs. Moreover, MiR-29a found in BMSCs-Exo could play a crucial role in preventing fibrosis during the process of endometrial repair in mice with IUAs. BMSCs-Exo overexpressing miR-29a can decrease the levels of α-SMA, Collagen I, SMAD2, and SMAD3, which are proteins involved in fibrotic signaling pathways [83]. BMSCs-Exos could be effective in IUA treatment by reversing EMT by transforming growth factor beta 1 (TGF-β1) downregulation. BMSC-Exos have a role in the healing process of damaged endometrium, exhibiting a comparable impact to BMSC [18]. Xiao et al. [84] showed that the exosomal transfer of microRNA-340 (miR-340) from BMSCs is involved in regulating the process of endometrium recovery. This process effectively inhibits the expression of collagen and α-smooth muscle actin (α-SMA) induced by TGF-β1, thereby preventing endometrial fibrosis in vitro.

Table 1. The effects of EVs derived From different sources of MSCs on IUAs

Author, Year	Animal Characteristic		Intervention				Assessment	Outcome	
	Animals Strain; Age	Disease Model	Source/Origin	Dose	Frequency; Time Point	Route	Methods	Molecular	Macroscopic
Tan Q et al. 2020 [83]	ICR mouse 8 weeks	1 mL syringe to insert the uterine cavity and injected 0.1 mL of ethanol	BMSCs- EVs + miR-29a	*	2 weeks	IU	RT-PCR: α SMA, Collagen I, SMAD2, and SMAD3 Staining: H and E	↓ α SMA Collagen I, ↓ SMAD2, ↓ SMAD3	↓ Fibrosis, ↑ Number of endometrial glands, ↑ Endometrial thickness
Yao et al. 2019 [18]	Female New Zealand white rabbits 4 weeks	Double injury	BMSC and BMSCs-Exo	5×10^5 BMSC 0.25 mL Exo (200 μg/mL)	1 week, 2 weeks, 3 weeks, and 4 weeks	IU (longitudinal muscle walls of the uterus)	H and E staining, Masson staining immunohistochemistry: CK19 and VIM Western blot: CK19, VIM, FSP-1, E-cadherin, TGF-β1, TGF-β1R, Smad 2, and P-Smad 2. RT-PCR:CK19, VIM, FSP-1, E-cadherin, TGF-β1, TGF-β1R, and SMAD 2	↑ CK19, ↓ vimentin, ↓ TGF-β1, TGF-β1R, and Smad2, ↓ Phosphorylation levels of TGF-β1, TGF-β1R, and Smad2, ↓ EMT	↓ Endometrial fibrosis area ↑ Number of endometrial glands
Xiao et al. 2019 [84]	Sprague-Dawley rats 8 weeks	Mechanical damage	BMSC-EVs + miR-340	500 μL	14 days	IU	Staining: H and E Trichrome, Real-time reverse-transcribed PCR: Collagen 1α1, α-SMA and (TGF)-β1, TGF-β1R	↓ Collagen 1α1, ↓ α-SMA, ↓ TGFβ1, ↓ TGF β1R	↑ Functional ↓ Fibrosis ↑ Number of endometrial glands ↑ Endometrial thickness
Liu et al. 2021 [85]	BALB/c mice 8 weeks	0.5 mg/kg LPS	BMSC-derived miR-223-3p-containing exosomes	10 μg exosoms per 100 μL	12 h	In vitro	Western blotting: NLRP3, ASC, N-Gasdermin D, cleaved caspase-3 and BAX RT-qPCR and ELISA: IL-18, IL-1β, IL-4, TNF-α	↓ IL-18 and IL-1β, TNF-α ↑ IL-4 ↓ Gasdermin D, NLRP3, ASC	↑ Cell viability ↓ Cell apoptosis
Yao et al. 2019 [18]	New Zealand white rabbits 4 weeks	LPS-soaked cotton	BBMSC-EVs	EVs: 25 μg/mL, 50 μg/mL, 100 μg/mL 5×10^5 BMSC	1 week 2 weeks 3 weeks 4 weeks	IU	Staining: H and E Trichrome Immunohistochemistry: CK19 and VIM Western blotting: CK19, VIM, FSP-1, E-cadherin, TGF-β1, TGF-β1R, Smad 2, and P-Smad 2 RT-PCR: CK19, VIM, FSP-1, E-cadherin, TGF-β1, TGF-β1R, and SMAD 2	↑ CK19, ↑ E-cadherin ↓ VIM ↓ TGF-β1 TGFβ1R ↓ SMAD2 ↓ FSP-1	↓ Fibrosis ↑ Number of endometrial glands no significant difference between BMSC and Exo treatment groups
Zhao et al. 2020 [27]	Sprague-Dawley rats 8 weeks	Mechanical curettage	ADSC- EVs	100 μg suspended in 0.2-mL PBS	2 weeks 4 weeks 8 weeks	IU	Staining: H and E Trichrome Western blot analysis: LIF, integrin-β3, VEGF	↑ LIF, ↑ Integrin-β3, ↑ VEGF	↓ Fibrosis ↑ Number of endometrial glands ↑ Endometrial thickness ↑ Number of implantation ↓ Time to conceive ↑Pregnancy rate

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Author, Year	Animal Characteristic		Intervention				Assessment	Outcome	
	Animals Strain; Age	Disease Model	Source/Origin	Dose	Frequency; Time Point	Route	Methods	Molecular	Macroscopic
Shao et al. 2021 [77]	Female mice 8-10 weeks	95% ethanol	ADSC-Exo	5 μg/mouse	Once	IU	Staining: H and E and Masson trichrome Western blot analysis: TGFβR1, α-SMA, and CK19 qPCR analysis: lncRNA-MIAT dual-luciferase reporter gene assay	↑Expression of lncRNA-MIAT α-SMA and TGFβR1 ↑ CK19 miR-150-5p expression repressed by lncRNA-MIAT	↓ Endometrial fibrosis
Zhang et al. 2021 [81]	Sprague-Dawley rats 10 weeks	Mechanical damage by syringe	MenSCs-EVs	4.25×10^{8} particles per mL, 300 μg mL−1	4.5 day 9 days 18 days	IU	Staining: H and E Trichrome Immunofluorescence: Ki-67 Western blot: LIF, ITGAV and VEGFA, collagen I, TGFβ1, p-SMAD3, SMAD3, p-ERK1/2 and ERK1/2	↑ Ki-67 ↑ LIF, ITGAV, and VEGFA ↓ collagen I ↓ TGFβ1, pSMAD3SMAD3, p-ERK1/2 ERK1/2	↓ Fibrosis ↑ Number of endometrial glands ↑ Endometrial thickness ↑ Number of embryos ↑ Size of the embryos
Zhang et al. 2021 [86]	18 infertile women with unresponsive thin endometrium	-	Collagen scaffold/ umbilical cord mesenchymal stem cells (CS/ UC-MSCs)	2×10^{7} UC-MSCs loaded onto a CS	In two consecutive menstrual cycles	Hysteroscopictransplantation into the uterine cavity	Histological analysis: H and E staining the expression of Ki67, ERα, and PR of the endometrium and Endometrial thickness, uterine receptivity, and endometrial angiogenesis, proliferation, and hormone response were compared before and after treatment. Pregnancy outcomes: endometrial receptivity	↑ Expression of Ki67, estrogen receptor α, and progesterone receptor	↑ Endometrial angiogenesis, proliferation, and response to hormones, number of glands
Li et al. 2023 [70]	Endometrial stromal cells	10 ng/mL TGFβ1	UCMSC-exos UCMSC-exos	20 μg/mL	Cells were harvested 48 h after transfection	In vitro	qRT-PCR: Fibrotic markers (α-SMA and COL1A1) Western blotting analysis: fibrosis-related markers α-SMA and COL1A1	↓ mRNA concentrations of fibrotic-associated Markers ↓ protein concentration of α-SMA and COL1A1	↓ TGFβ1- induced endometrial fibrosis
Shi et al. 2021 [71]	Endometrial stromal cells	60 μmol/L mifepristone	UCMSC-Exos	UCMSCs were seeded on top of the artificial basement membrane of insert chambers	-	In vitro	Deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining qRT-PCR: The miRNA microarray analysis. Prediction of miRNA-mRNA interactions The miRNA mimics/inhibitor transfection flow cytometry analysis for cell apoptosis Western blotting. Dual-luciferase reporter assay	Shuttling of miR-7162-3p	Protected ESCs from mifepristone-induced apoptosis repairing the damaged ESCs

Author, Year	Animal Characteristic		Intervention				Assessment	Outcome	
	Animals Strain; Age	Disease Model	Source/Origin	Dose	Frequency; Time Point	Route	Methods	Molecular	Macroscopic
Wang et al. 2023 [87]	40 female Sprague-Dawley rats (8–12 weeks old) human stromal cells	95% ethanol 10 ng/mL TGF-β1	hucMSC-sderived exosomal microRNA-202-3p (miR-202-3p)	100 μg/rat	Post endometrial injury (The rats were sacrificed on day 3 and day 7 after surgery)	Femoral vein	PCR Western blotting Staining: H and E, Mason's trichrome, Sirius red (for collagen fibers)	↓ MMP11 miRNA level ↑ COL1A1, COL3A1, COLVI, and fibronectin (FN) protein in vivo and in vitro	↑ Repair of Injured Endometrium
Yuan et al. 2023 [88]	Human endometrial epithelial cells BALB/c female mice (8-week-old)	10 ng/mL TGF-β1 Dual damage	UCMSCs-derived exosomal miR-543	5 μg/mouse	21 days of treatment	IU	RT-qPCR assay N-cadherin, α-SMA, fibronectin 1 Western blotting Immunohistochemistry (IHC) assay	↓ Expressions of N-cadherin, α-SMA, fibronectin 1 ↑ Expression of E-cadherin in TGF-β1-treated hEECs. in vivo, N-cadherin, α-SMA and fibronectin 1 protein expressions	↓ EMT in hEECs exposed to TGF-β1 ↓ Endometrial fibrosis
Saribas et al. 2020 [75]	Wistar albino rats 8 weeks	Scraping the uterine horn by needle	Rat UMSC UMSC-EVs	2×10^6 UMSC 2×10^6 UMSC-Exo	8 weeks	IU	Staining: H and E Trichrome Immunohistochemistry: MMP-2, MMP-9, TIMP-2 Immunohistochemical anti-BrdU Staining	↑ MMP-9 ↑ MMP2 ↓ TIMP-2	↓ Fibrosis, collagens, inflammation in the endometrium, and adhesion. ↑ Number of endometrial glands
Xin et al. 2020 [76]	Sprague-Dawley rats 9 weeks	Scraping completely using a T10 scalpel blade	hUCMSCs-EVs on scaffolds	3×10^{11}/mL	0 day 7 days 30 days 60 days	IU	Immunohistochemistry: ER, PR Staining: H and E Trichrome Immunofluorescent images of macrophages stained by F4/80 and CD86/CD163 ELISA: IL-1β, IL-6, TNF-α, IL-10, TGF-β, and VEGF-B	↓ TGFβ, ↓ IL-6, ↓ IL-1β, ↓ TNFα, ↓ CD163, ↓ CD86, ↑ ESR, ↑ PR, ↑ VEGF ↑ F4/80M	Endometrium regeneration collagen remodelling ↑ Expression of the estrogen receptorα/progesterone receptor ↑ Fertility facilitated M2 macrophage

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Author, Year	Animal Characteristic		Intervention				Assessment	Outcome	
	Animals Strain; Age	Disease Model	Source/Origin	Dose	Frequency; Time Point	Route	Methods	Molecular	Macroscopic
Jiang et al. 2022 [74]	C57BL/6 8 weeks	0.1 mL ethanol	UCMSC- EVs	1.25 mg/mL, 300 µL	6 h 12 h 24 h 7 day	IV	Immunohistochemical: Ki-67, CD31 Western blotting: Bcl-2, Bax, Cleaved Caspase-3 PTEN, AKT, and p-AKT RT-PCR: VEGF, IGF-1	↓ VEGF, IGF-1 ↑ CD31 ↓ Bcl2, ↓Bax, Cleaved Caspase-3 ↑ Ki-67	↑ Reproductive function ↓ Fibrosis ↑ Number of endometrial glands ↑ Endometrial thickness ↑ Fertility restoration, ↑ Embryo implantation
Liu et al. 2024 [89]			Exosomes derived from placental MSCs (miR-125b-5p, miR-30c-5p, and miR-23a-3p, enriched in PMSC-exosomes)					↑ Cell proliferation and endometrial thickness ↓ Fibrosis ↓ TGF-β/smad signaling pathway ↓ Expression of smad2 and smad3	↑ Repair endometrial damage and fertility in injured animals
Li et al. 2022 [82]	Balb/c mice 8 weeks	Electric tool-scratching	Tumor necrosis factor-a-primed MSCs-derived exosomes	25 mg/mL 1×10^6 cells in 200 mL of PBS	7 days	IV	Staining: H and E Trichrome ELISA and qRTPCR: IL-1b, IL-6, and TNF- α Immunohistochemistry: α SMA flow cytometry: CD86/CD163, F4/80, CD206	↑ CD163, F4/80, CD206 ↓ IL-1b, IL-6, and TNFα ↓ αSMA ↓ CD86	↑ Macrophage polarization to M2 ↓ Collagen ↓ Fibrosis ↑ Endometrial thickness
Lin et al. 2021 [90]	Sprague-Dawley rats 8 weeks	Anhydrous ethanol	ADSC- EVs + hydrogel	0.7×10^5	7 days	IU	Immunohistochemical staining: CD31 α-SMA collagen qRTPCR: HOXA-1, LIF, ER, PR, Integrin β3, IGF-1, VEGF, bFGF Western blotting: LIF, VEGF, and IGF-1	↑ CD31 ↓ αSMA, collagen ↑ LIF ↓ VEGF, IGF-1, bFGF ↑ ER, PR ↓HOXA	↓ Fibrosis ↑ Number of endometrial glands ↑ Endometrial thickness ↑ Fertility restoration ↑ Embryo implantation

Author, Year	Animal Characteristic		Intervention				Assessment	Outcome	
	Animals Strain; Age	Disease Model	Source/Origin	Dose	Frequency; Time Point	Route	Methods	Molecular	Macroscopic
Zhu et al. 2022 [78]	Sprague-Dawley rats 8 weeks	95% ethanol	BMSC- EVs CTF1-over-expressing BMSCs-EVs	100 µg/mL in a hydrogel suspension (20 µg in 200 µL hydrogel in each horn)	8 weeks	IU	Staining: H and E Trichrome immunofluorescent staining: CD31 and α-SMA Western blot: JAK, p-JAK,PI3K, p-PI3K, AKT, p-AKT, mTOR, p-mTOR, STAT3, and p-STAT3 Flow Cytometry CD29, CD34, CD44, CD45, and CD90	↑ CD31 ↑α-SMA ↑ JAKp-JAK,PI3K, p-PI3K, AKT, p-AKT, mTOR, p-mTOR, STAT3, and p-STAT3	↓ Fibrosis Endometrial thickness ↓ Collagen ↑ Migration ↑ Neovascularization myometrial regeneration ↑ Embryo implantation

Abbreviations: BMSC: Bone marrow mesenchymal stem cells; EVs: Extracellular vesicles; IU: Intrauterine; RT-PCR: Reverse transcription polymerase chain reaction; H and E: Hematoxylin and eosin stain; EM: Epithelial-mesenchymal transition; LPS: Lipopolysaccharide; ELISA: Enzyme-linked immunosorbent assay; IL: Interleukin; TNF: Tumor necrosis factor; ADSC: Adipose-derived mesenchymal stem cells; PBS: Phosphate-buffered saline; UCMSC: Umbilical cord mesenchymal stem cells; qRT-PCR: Quantitative real-time polymerase chain reaction.

Adipose tissue-derived EVs

Adipose-derived MSCs (ADSCs) are found in adipose tissue and have a superior potential to regenerate themselves compared to other types of MSCs. They can be collected simply and safely through outpatient liposuction operations [94]. ADSCs' high level of accessibility enables the success of an autologous stem cell transplantation with minimal ethical concerns and the lowest risks of immune rejection [95, 96]. Thus, ADSC is a highly suitable candidate for clinical translation [97]. Zhao et al. demonstrated that the administration of exosomes derived from ADSCs (ADSC-Exos) resulted in the preservation of the typical uterine structure, facilitated the process of endometrial regeneration and collagen reorganization, and augmented the level of vascular endothelial growth factor (VEGF) expression. The results of their study indicated that the administration of ADSC-Exos facilitated the process of endometrial regeneration and contributed to the restoration of fertility in the mouse model [27]. Moreover, in Shao et al.'s study [77] both in vivo and in vitro experiments demonstrated that ADSC-Exo treatment effectively inhibited endometrial fibrosis. This was evidenced by the reduction in the expression of hepatic fibrosis markers (α-SMA and TGF-βR1) and the increase in the expression of cytokeratin 19 (CK19). Additionally, this study showed that long noncoding RNA-myocardial infarction-associated transcript (lncRNA-MIAT) up-regulation might be a potential mechanism of ADSC and ADSC-Exo in their therapeutic effects.

Menstrual blood-derived EVs

Menstrual blood-derived stem cells (MenSCs) are a type of MSC originating from the endometrium and shed during menstruation. These cells are easily accessible, obtained without causing harm to the donor, and align with ethical principles. The benefits above suggest the possibility of extensive utilization of MenSCs in the field of regenerative medicine [98, 27]. Zhang et al. demonstrated that similar to the MenSCs, small EVs derived from MenSCs (MenSCs-sEVs) successfully restored the structure of the uterus, stimulated the regrowth of glands and blood vessels, and reversed the formation of fibrotic tissue. Repeated transplantation of MenSCs-sEVs significantly improved endometrial receptivity and pregnancy outcome. MenSCs tended to migrate through the bloodstream, while MenSCs-sEVs had a more localized therapeutic effect. In addition, MenSCs and MenSCs-sEVs were found to inhibit the TGFβ1/SMAD3 signaling pathway in the IUAs endometrium while stimulating the phosphorylation of SMAD1/5/8 and extracellular signal-regulated kinases 1/2 (ERK 1/2) and increasing the expression of BMP7 anti-fibrogenic gene. These findings suggest that MenSCs-sEVs have the potential to safely and effectively improve endometrial restoration, making them a promising non-cellular therapy for endometrial regeneration in the context of IUAs. However, the results indicate that MenSCs-sEVs need to be administered repeatedly for therapeutic purposes, highlighting the need to explore ways to enhance the yield or in vivo durability of MenSCs-sEVs [81].

Umbilical cord-derived EVs

Umbilical cord MSCs (UCMSCs) are considered an ideal source of MSCs, making them desirable for therapeutic applications [99, 100]. UCMSCs exhibit a greater ability for differentiation, migration, and self-renewal compared to other types of MSCs. Additionally, they can be obtained without invasive procedures [101]. Mounting evidence suggests that UCMSCs play a significant role in restoring the injured endometrium [102]. For instance, the transplantation of UCMSCs in a rat model has been shown to restore the thickness of the endometrium and reduce excessive fibrosis [103]. UCMSCs augment the reaction of the endometrium to hormones and boost both endometrial proliferation and angiogenesis in patients struggling with thin endometrium. In a study conducted by Zhang et al., transplantation of collagen scaffold/UCMSCs resulted in increased endometrial thickness. Additionally, of 15 patients, three of them got pregnant after frozen-thawed embryo transfer [86]. Furthermore, exosomes derived from UCMSCs (UCMSCs-Exos) have shown therapeutic effects on IUAs. For example, UCMSC-Exosomal miR-145-5p improved the condition of endometrial fibrosis produced by TGF-β1. One of the mechanisms underneath observed fibrosis prevention appears to be targeting zinc finger E-box-binding homeobox 2 (ZEB2) expression by Exosomal miR-145-5p [70]. In another investigation, UCMSCs-Exos demonstrated a reduction in mifepristone-induced death of endometrial stromal cells. Additionally, three specific miRNAs (miR-6831-5p, miR-4669, and miR-7162-3p) were considered potential agents responsible for the UCMSC-Exos-mediated restoration of the endometrium. The researchers demonstrated that miR-7162-3p was transported by Exos from UCMSCs and controlled the expression of apolipoprotein L6 (APOL6) by specifically targeting its 3'-UTR in endometrial stromal cells (ESCs). Therefore, the UCMSC-Exos that overexpress miR-7162-3p can potentially be utilized in cell-free therapy for endometrial damage [71]. Another study was conducted to examine the effect of UCMSC-Exosomal microRNA-202-3p (miR-202-3p) on repair-

ing endometrial injury. The researchers suggested that the overexpression of miR-202-3p in exosomes played a role in promoting the early repair of injured endometrium by regulating ECM remodeling. Exosomal miR-202-3p derived from UCMSCs showed the ability to control the expression of matrix metalloproteinase 11 (MMP11) and enhance the buildup of ECM components, including collagen 1A1 (COL1A1), COL3A1, COLVI, and fibronectin, during the initial phase of endometrial injury repair [87]. Furthermore, miR-543 has the potential to be transmitted from UCMSCs to human endometrial epithelial cells (hEECs) by exosomes. Exosomal miR-543 effectively decreased the levels of N-cadherin, α-SMA, and fibronectin 1, while increasing the expression of E-cadherin in TGF-β1-treated hEECs [88].

Placenta-derived EVs

Exosomes produced from placental MSCs (PMSCs-Exos) can restore uterine function and increase fertility in mice. This is accomplished by stimulating cell proliferation, increasing endometrial thickness, and reversing fibrosis. In terms of the molecular mechanism underlying these therapeutic effects, Liu et al. identified three specific miRNAs, miR-125b-5p, miR-30c-5p, and miR-23a-3p, which are enriched in PMSC-Exos, as significant participants in the treatment of IUAs. miR-125b-5p/miR-30c-5p and miR-23a-3p suppressed SMAD2 and SMAD3 expression by targeting their 3'-untranslated regions, leading to downregulation of the TGF-β/SMAD signaling pathway and fibrosis reversal. Notably, the safety of PMSC-exosomes during intrauterine treatment has been verified. Exosomes from PMSCs can repair endometrial damage and improve fertility in damaged mice via modulating the TGF-β/SMAD pathway through miR-125b-5p, miR-30c-5p, and miR-23a-3p. This provides insights into the precise treatment of IUA using exosome-based cell-free therapy [89].

Discussion

Innovative and combinational approaches

This study reviews articles that aimed to enhance the effects of MSC-EVs on IUAs by loading EVs on biological scaffolds or other innovative approaches. In a rat endometrium-damage model, Xin et al. created a construct of exosomes and collagen scaffold (CS/Exos) for endometrial regeneration and examined the regeneration mechanism via macrophage immunomodulation. The CS/Exos transplantation significantly improved endometrial regeneration, collagen remodeling, estrogen receptor/progesterone receptor expression, and fertility. In vivo and in vitro, CS/Exos facilitated CD163+ M2 macrophage polarization, decreased inflammation, and boosted anti-inflammatory responses. According to RNA-seq, exosome-enriched miRNAs were the primary mediators of exosome-induced macrophage polarization [76]. Therefore, inducing M2 macrophage polarization as a repair facilitator and anti-inflammatory agent seems feasible in treating IUAs. In another study, Li et al. found that exosomes derived from UCMSCs pre-treated with tumor necrosis factor-α (TNF-α) can polarize macrophages into M2 phenotype in a mouse model of IUAs. They suggested that the increased expression of Galectin-1 in the exosomes affects the Jak-STAT signaling pathway and thus induces the M2 phenotype [82]. In an intriguing study by Lin et al. [90], a novel approach was employed to treat IUAs by utilizing an exosome-hydrogel construct. The researchers developed a multifunctional microenvironment-protected exosome-hydrogel for in situ microinjection and endometrial regeneration, aiming to restore fertility. By employing Ag+-S dynamic coordination and fusion with ADSC-Exo, they created an injectable preparation that effectively reduced infection risk while maintaining the antigenic contents and paracrine signaling activity of the ADSC source cells. This innovative exosome-hydrogel construct demonstrated its potential by promoting neovascularization in vitro, leading to a significant increase in human umbilical vein endothelial cell proliferation and tube formation. Notably, the exosome-hydrogel construct not only protected the surrounding microenvironment but also stimulated blood vessel formation and tissue regeneration in vivo while reducing fibrosis in the affected areas. This comprehensive endometrial regeneration strategy improved receptivity to embryo implantation. This next study examined how exosomes derived from BMSCs overexpressing cytokine corticotrophin-1 CTF1 (C-BMSCs) affect fertility outcomes in the rat model of IUAs. Compared to BMSCs-Exos and model control groups, they discovered that these C-BMSCs-Exo promoted superior endometrial regeneration. Endothelial cell proliferation, migration, and tube formation were also increased in vitro and in vivo after treatment with Exos. C-BMSCs-Exo treatment also improved endometrial healing and increased angiogenesis in vitro and in vivo compared to BMSCs-Exo treatment. The researchers also discovered that the JAK/PI3K/mTOR/STAT3 signaling pathways were upregulated in rats treated with C-BMSC Exo, which may highlight the molecular mechanism underlying CTF1's beneficial effects on endometrial angiogenesis and regeneration. Their findings highlighted a potential technique to treating injured endometrial tissues by demonstrating that CTF1 can promote angiogenesis, reduce tissue fibrosis, and enhance endometrial receptivity after BMSCs-Exo therapy [78].

Conclusion

In conclusion, MSC-derived EVs represent a promising avenue for the treatment of IUAs. EVs derived from different sources of MSCs, including bone marrow, adipose tissue, umbilical cord, menstrual blood, and placenta, show different degrees of ability in decreasing fibrosis area, increasing the number of endometrial glands, and, most importantly, restoring fertility in animal models of IUAs. Besides MSC-EVs showed potential in reversing EMT and downregulation of fibrotic markers in vitro. Although the exact mechanism behind the therapeutic effects of MSC-EVs is yet to be explored, the role of miRNAs, as a key component of EVs, on fibrotic signaling pathways appears to be critical. Enriching EVs with specific miRNAs, loading them on biological constructs, or combining them with other therapeutic approaches like hormone or cytokine therapy might enhance the effectiveness of EV treatment. Furthermore, pre-treating MSCs with different biological compounds to enhance the EVs therapeutic potential or utilizing MSC-EVs to polarize M1 macrophages to suppress fibrosis in IUAs are among the innovative treatment approaches. Future studies could shed light on the molecular mechanisms beneath MSC-EVs potency in IUAs or investigate this treatment in a clinical setting.

Ethical Considerations

Compliance with ethical guidelines

The authors confirm this research complies with all relevant ethical guidelines and regulations. No ethical concerns or are associated with this work.

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Authors' contributions

Conceptualization and supervision: Seyed Mahmoud Hashemi; Data curation, investigation and writing the original draft: Mir Mohammadreza Hosseini and Kimiya Rashidan; Review and editing: Ashkan Rasouli-Saravani and Seyed Mahmoud Hashemi.

Conflicts of interest

The authors declared no conflict of interest.

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