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REVIEW ARTICLE

Exosomes derived from mesenchymal stromal cells: a promising treatment for pelvic floor dysfunction

Leimei Xu[1,2] · Yizhen Sima[1,2] · Chengzhen Xiao[1] · Yisong Chen[1,2]

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Abstract

Pelvic floor dysfunction (PFDs), which include pelvic organ prolapse (POP), stress urinary incontinence (SUI) and anal incontinence (AI), are common degenerative diseases in women that have dramatic effects on quality of life. The pathology of PFDs is based on impaired pelvic connective tissue supportive strength due to an imbalance in extracellular matrix (ECM) metabolism, the loss of a variety of cell types, such as fibroblasts, muscle cells, peripheral nerve cells, and oxidative stress and inflammation in the pelvic environment. Fortunately, exosomes, which are one of the major secretions of mesenchymal stromal cells (MSCs), are involved in intercellular communication and the modulation of molecular activities in recipient cells via their contents, which are bioactive proteins and genetic factors such as mRNAs and miRNAs. These components modify fibroblast activation and secretion, facilitate ECM modelling, and promote cell proliferation to enhance pelvic tissue regeneration. In this review, we focus on the molecular mechanisms and future directions of exosomes derived from MSCs that are of great value in the treatment of PFD.

Keywords Exosomes (EXs) · Mesenchymal stromal cell (MSC) · Pelvic floor dysfunction (PFDs) · Fibroblast · Extracellular matrix (ECM)

Abbreviations

PFD	Pelvic floor disorders
POP	Pelvic organ prolapse
SUI	Stress urinary incontinence
AI	Anal incontinence
ECM	Extracellular matrix
MSC	Mesenchymal stromal cell
AI	Anal incontinence
PFMT	Pelvic floor muscle physiotherapy
BF	Biofeedback
FBR	Foreign body response
FDA	Food and drug administration
EMA	European medicines agency
HIF-1α	Hypoxia-inducible factor 1α
AGEs	Advanced glycation end products
MMPs	Matrix metalloproteinases
TIMPs	Tissue inhibitors of matrix metalloproteinases
OS	Oxidative stress
MnSOD	Mitochondrial superoxide dismutase
GPX	Glutathione peroxidase
EVs	Extracellular vesicles
TEM	Transmission electron microscopy
NTA	Nanoparticle tracking analysis
HSP70	Heat shock protein 70
TSG101	Tumour-susceptibility gene 101
MVBs	Multivesicular bodies
SNAREs	Soluble N-ethylmaleimide-sensitive factor attachment protein receptors
ESCRT	The endosomal sorting complex required for transport
MSCs-Ex	Mesenchymal stromal cell-derived exosomes
HUCMSCs	Human umbilical cord mesenchymal stromal cells
LPP	Leak point pressure
eMSCs	Human endometrial mesenchymal stromal cells
BMSCs	Bone marrow mesenchymal stromal cells
ADSC	Adipose-derived stromal cell
USC	Urine-derived stromal cell (USC)

✉ Yisong Chen
cys373900207@163.com

[1] Department of Gynecology, Obstetrics and Gynecology Hospital of Fudan University, 128 ShenYang Road, Shanghai 200011, People's Republic of China

[2] Shanghai Key Laboratory of Female Reproductive Endocrine-Related Diseases, Shanghai, China

SCs	Satellite cell
ERK	Extracellular-regulated protein kinases
SIRT1	Silent mating type information regulation 2 homologue 1 (SIRT1)
DRG	Dorsal root ganglion
SMC	Smooth muscle cell
AIF	Apoptosis-inducing factor
PARP-1	Upregulating poly ADP-ribose polymerase 1
PAR	Poly ADP-ribose
JAG1	Jagged1
ROS	Reactive oxygen species
Nrf2	Nuclear factor-E2-related factor 2
TLR4	Toll-like receptor 4
dECM	Decellularized scaffold ECM
TFF	Tangential flow filtration

Introduction

Pelvic floor dysfunction (PFD) describes a series of clinical diseases such as include pelvic organ prolapse (POP), stress urinary incontinence (SUI) and anal incontinence (AI), which have dramatic effects on the well-being and satisfaction of millions of adult women [1].

In addition to the reduction in various types of cells, such as fibroblasts, smooth muscle cells and neural cells, in pelvic tissues, PFD develops as a result of qualitative or quantitative defects in pelvic connective tissues, including ligaments, fascia, and the levator ani, urethra and anal sphincter, which cannot provide sufficient support for the pressure from the abdominal cavity [2, 3]. Therefore, any biological event that interferes with the functional capacity of connective tissue or its repair process, as well as the depletion of any pelvic component without adequate supplementation, may promote the occurrence and development of PFD [4, 5].

Currently, the treatments for PFD can be divided into nonsurgical and surgical therapies, which are still conservative and based on symptoms. The former mainly includes pelvic floor muscle physiotherapy (PFMT), biofeedback (BF) and electronic stimulation, which can alleviate symptoms but fail to restore anatomic structures and have a high recurrence rate of up to 20–30% [6, 7]. The latter could help to achieve better anatomical and functional restoration but is concomitant with many medical complications [7–10], such as foreign body response (FBR), scarring, chronic pain and especially erosion in the mesh implantation site [11–14]. Because of the warnings of the Food and Drug Administration (FDA) and European Medicines Agency (EMA), many types of meshes have been banned from gynaecologic surgical operations [12]. Thus, alternative treatments to promote the regeneration and repair of damaged tissue are urgently needed.

Recently, the use of mesenchymal stromal cells (MSCs) has become a promising therapeutic approach for PFD and regenerative medicine and has attracted much interest because of the various capacities of these cells, such as self-renewal, multipotency, immunoregulation and secretory functions [5, 15, 16]. Under specific conditions, MSCs can migrate to the damaged site and differentiate into certain local cells to replace the lost, senescent, apoptotic and diseased cells and accelerate the repair progress. Furthermore, MSCs have powerful antiapoptotic, anti-inflammatory and neuroprotective effects. Although it has been gradually studied and appreciated (Table 1), stem cell therapy has many limitations, such as the heterogeneity of progeny stem cells, cell ageing, potential tumorigenesis, immune rejection and thrombosis [17, 18], which hinders its use in human regenerative medicine [19, 20]. Therefore, finding a new biological product to replace MSC therapy is an essential research subject due to these limitations.

MSCs promote tissue repair and regulate immunity primarily via paracrine factors instead of differentiation. Exosomes, which are one of the major secretory products of MSCs, are considered a cell-free approach that is superior to stem cell transplantation [21–23]. Accumulating evidence suggests that stem cells can mediate tissue regeneration and functional improvements via paracrine effects, rather than undergoing de novo differentiation, as confirmed by the administration of conditioned medium containing the secreted factors inducing equivalent beneficial effects. Compared to local exosome administration, MSC administration without exosomes delayed wound healing and decreased M2 macrophage polarization [24]. Exosomes reduce the risk of cell-based therapies, such as tumorigenesis, thrombosis and malformation, as mentioned previously. In addition, exosomes are convenient to collect and store and have low immunogenicity. Furthermore, they are enriched with various kinds of molecular cargo (such as mRNAs, miRNAs, and proteins) that affect numerous biological processes in target cells [25]. In summary, exosomes provide a safer and more efficient novel treatment with broader prospects than MSCs.

This review is intended as an overview of the effects and mechanisms of MSC-derived exosomes (MSC-Exs) in PFD, as well as future directions (Fig. 1).

The mechanisms of PFD

Pelvic floor connective tissues mainly consist of fibroblasts and extracellular matrix (ECM), which is the product of

Table 1 MSC-based therapies for PFD

Condition	Model	Cell type	Treatment	Results	References
Bilateral ovariectomy	Rat	HUCMSCs	Subepithelial injection	Normalized the fibromuscular structures of the vagina	[92]
Ovariectomy	Rhesus monkey	HUCMSCs	Vagina was implanted with SIS grafts seeded with HUCMSCs	Significantly promoted the regeneration of ECM, smooth muscle bundles and vascularization	[93]
Subcutaneous rat model of wound repair	Rat	eMSCs	Scaffolds seeded with eMSCs	Enhanced collagen growth and organization	[94]
SUI	Rat	BMSCs	Periurethral injection	The restoration of LPP	[95]
SUI	Rat	BMSCs	Periurethral injection	restored the damaged external urethral sphincter	[96]
POP	In vitro	BMSCs	Combination of electrospun core–shell nanofibers of poly(l-lactic acid)-hyaluronic acid (PLLA/HA) and BMSCs	Improved cellular function in stem cells in the composite nanofibers	[97]
PFD	Rat	BMSCs	Injection of PLGA-loaded bFGF-NPs and BMSCs	Promoted the outcome of urodynamic tests	[98]
PFD	Rat	BMSCs	Downregulation of the endogenous microRNA-29a-3p in BMSCs	Increased expression and secretion of elastin and improved outcomes of the urodynamic test results	[99]
POP	In vitro	ADSCs	Stable overexpression of basic fibroblast growth factor (bFGF) in ADSCs	Increased pelvic reconstruction and fibroblast growth	[100]

Human umbilical cord mesenchymal cells (*HUCMSCs*); Leak point pressure (*LPP*); Human endometrial mesenchymal stromal cells (*eMSCs*); Bone marrow mesenchymal stromal cells (*BMSCs*)

fibroblasts and contains type I and III collagen fibres, elastin, fibulins, fibronectin, laminins, hyaluronic acid and a variety of glycoproteins [26, 27]. The major components of the ECM are collagen I and collagen III; the former provides tension, while the latter is important for the elasticity and resilience of connective tissues. Fibroblasts can remodel the ECM to maintain the pelvic microenvironment through the production of collagens and the activation of catabolic enzymes (e.g. matrix metalloproteinases (MMPs)) [28].

Defects in the number and function of pelvic fibroblasts

Unfortunately, several studies have confirmed that the morphology and essential functions of the fibroblasts of PFD patients are severely damaged, which results in the gradual weakening of supportive tissues. Chen found that POP patient fibroblasts may be more likely to have fewer organelles, more irregular shapes [29], higher apoptosis rates [30, 31] and large declines in adhesion capacity, collagen gel shrinkage, mechanical reactivity and collagen secretion [28, 32–34]. There have been insights into the overexpression of apoptotic proteins, including cytochrome c, bax, bad, caspase 3, caspase 9 [35–38] and hypoxia-inducible factor 1a (HIF-1a), which can mediate fibroblast apoptosis through death receptor and mitochondrial apoptotic pathways [39].

Chen provided direct evidence of an increase in advanced glycation end products (AGEs) in POP sites, which showed that the activation of AGE receptors could trigger the downstream MAPK/NF-κB signalling pathway to suppress cell proliferation [40].Similarly, there is reduced cellularity and less muscle content in prolapsed pelvic tissue than in women with normal pelvic support [41–43]. The main reasons for SUI may be injuries to the pubococcygeal muscle [44] and pudendal nerve [45].

Disorganized ECM proteins

Because of the decreased number of fibroblasts with normal functions, collagen fibres and elastin have great quantitative and qualitative defects in the tissues of PFD patients [27, 29, 31, 32, 46–50]. ECM degradation is precisely regulated by MMPs and their endogenous suppressors, tissue inhibitors of matrix metalloproteinases (TIMPs) [51, 52], and these processes involve a variety of signalling pathways [53, 54]. Studies have shown that MMP1 and MMP3 expression are increased [31, 55, 56] and TIMP1 and TIMP2 expression are decreased [50, 57, 58] in PFD patient tissues.

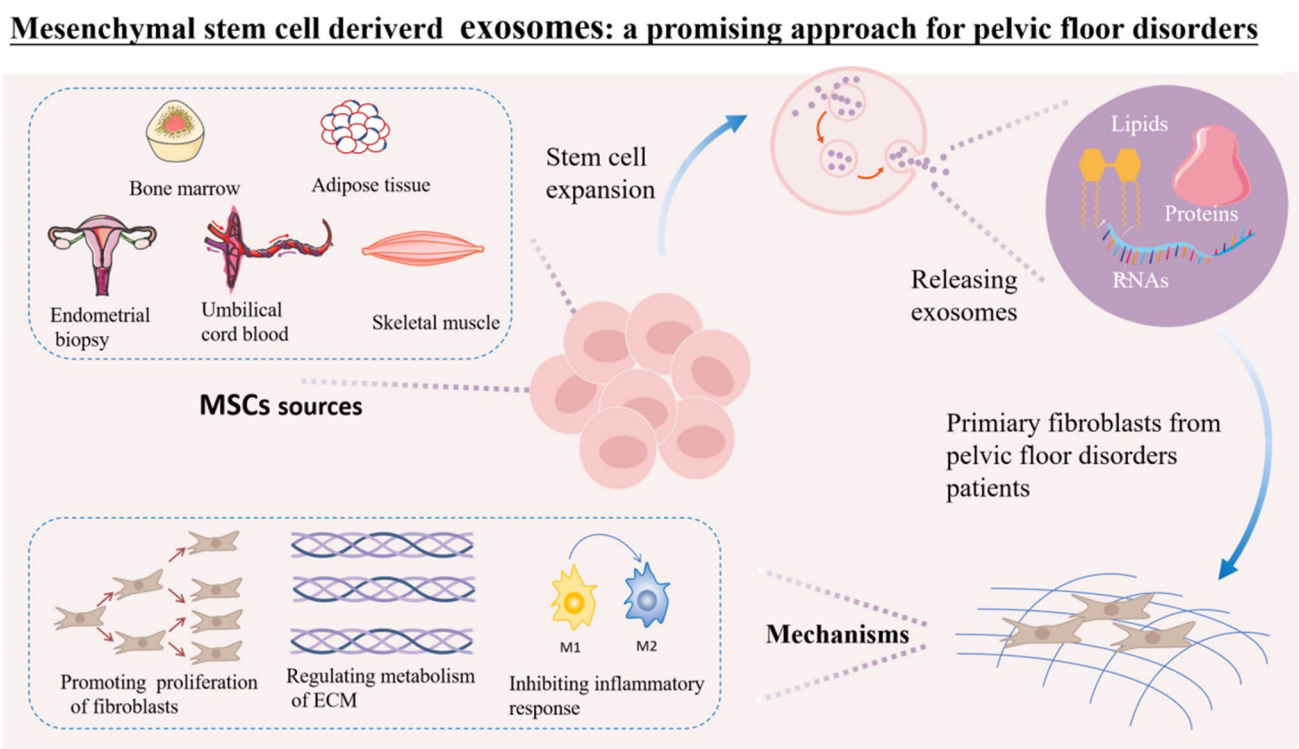

Fig. 1 Exosome-mediated reconstruction of the pelvic floor environment. MSCs from various sources (bone marrow, adipose tissue, endometrial biopsy, umbilical cord blood, skeletal muscle, etc.) can promote the function of fibroblasts and regulate immunity by releasing exosomes. These extracellular vesicles contain lipids, proteins and RNAs, which could promote the proliferation of fibroblasts and modulate the metabolism of ECM, as well as regulate immunity

Pelvic oxidative stress (OS) and inflammatory imbalance

Many chronic diseases share the feature of complex interactions between OS and inflammatory imbalance. Increasing lines of evidence have demonstrated that OS and inflammation are widespread in the pelvic floor. For instance, antioxidant proteins, including mitochondrial superoxide dismutase (MnSOD) and glutathione peroxidases (GPX1, GPX3) [59, 60], are downregulated, whereas there is an opposite trend in molecules related to inflammation (cyclooxygenase 2 and prostaglandin E2) [58, 61] and OS (8-OHdG and 4-HNE) [39]. It was previously suggested that changes in the synthesis of ECM occur in fibroblasts treated with hydrogen via the TGF-β signalling pathway [62]. Moreover, Hong showed that mechanical stress can promote intracellular reactive oxygen species (ROS) levels and decrease mitochondrial membrane potential, which indicates that mechanical stress leads to the development of PFD via intracellular OS [63]. Wu demonstrated that t high concentrations of COX-2 could improve the level of PGE2, resulting in the upregulation of MMP1 expression [64]. Thus, OS participates in PFD by influencing the activities of fibroblasts and collagen metabolism [39, 59, 60, 62, 63, 65].

These findings support the connective tissue injury theory that impaired fibroblasts and muscles and an imbalance in ECM metabolism result in supportive connective tissue weakness [28], eventually causing PFD [66].

Exosomes

Extracellular vesicles (EVs) were first reported by E Chargaff in 1946 [67], and secretory membrane fragments were shown to be a universal phenomenon in 1977 [68]. Harding and Johnstone pronounced that transferrin receptors were released into the ECM during reticulocyte maturation via vesicles (50 nm in diameter), which were later named exosomes [69–71]. To date, EVs have been divided into three types: apoptotic bodies (800–5000 nm in diameter), microvesicles (200–1000 nm in diameter) and exosomes (30–150 nm in diameter) depending on size, contents, and mechanism of formation [72]. The first two vesicle types

Table 2 Studies in which exosomes or EVs have been used to treat PFD

Condition	Model	Cell source	Results	References
SUI	Rat	iPSCs	Alleviation of urethral LPP and tissue structure in the SUI rat	[101]
SUI	In vitro	M2 macrophages	Repair of damaged pubococcygeal muscle by promoting myotube formation, myoblast differentiation and improvements in inflammatory cell infiltration	[102]
SUI	Rat	ADSCs	Improved function and histological recovery of the urethra caused by ADSC-EXs by promoting the proliferation of skeletal muscle and Schwann cells	[21]
SUI	Rat	USCs	The recovery of injured muscle tissue and urethral function by the activation, proliferation and differentiation of SCs mainly via the phosphorylation of ERK	[22]
SUI	Rat	BMSCs	Induction of the proliferation, differentiation and activation of SCs via BMSC-Exs overexpressing SIRT1	[23]
SUI	Rat	ADSCs	Increased collagen levels by inhibiting degradation and enhancing the synthesis in vaginal fibroblasts	[103]
Mesh exposure model	Pig		Increased epithelial thickness and capillary density after local injection of exosomes	[104]
DRG cells	In vitro	Schwann cell lines	Facilitating the proliferation and inhibiting apoptosis and senescence in injured DRG cells	[105]

MSC (MSC), adipose-derived stromal cell (*ADSC*), urine-derived cell (*USC*), satellite cells (*SCs*), extracellular-regulated protein kinases (*ERK*), silent mating type information regulation 2 homologue 1 (*SIRT1*), dorsal root ganglion (*DRG*)

originate from the plasma, and the third is endogenous [73, 74]. Exosome contents can not only indicate their origins but also be passed to other cells as messengers to change cell functions [21–23, 75]. Exosomes were regarded as cellular waste in the past; however, the contents carried in exosomes play major roles in cellular activities and pathological processes, including the immune response, angiogenesis, cell death, neurodegenerative diseases and cancers [76, 77]. Exosomes are also one of the major secretory products of MSCs and are considered a cell-free approach that is superior to stem cell transplantation.

The formation of exosomes

The identification of exosomes is of great importance and could indicate their sources and biological functions. Well-recognized methods vary from physical properties to biochemical characteristics, such as transmission electronic microscopy (TEM), nanoparticle tracking analysis (NTA) and Western blotting, to confirm exosome morphology, size, porosity and surface markers [78, 79]. NTA, one of the key biophysical technologies, can measure exosome concentrations and size distribution by tracking the Brownian motion of individual vesicles. Moreover, NTA can also determine exosome phenotype through the association of fluorescent measurements [80]. Transmembrane proteins (such as CD9 and CD63) and some molecules associated with biological functions, including heat shock protein 70 (HSP70) and tumour-susceptibility gene 101 (TSG101), are common components used to identify exosomes, which can be detected by Western blot analysis [81].

Exosome biogenesis, which is tightly regulated, consists of three main stages: (1) endocytic vesicle generation via invagination of the plasma membrane; (2) formation of multivesicular bodies (MVBs) via inward budding of the endosomal membrane; and (3) fusion of these MVBs with the plasma membrane to release internal vesicles as exosomes [82–85]. Exosomes are protected from degradation by ribonucleases by their lipid bilayer and communicate with other cells with the help of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) and the endosomal sorting complex required for transport (ESCRT) [85, 86]. As a type of messenger, exosomes play a significant role in intercellular communication, which is mainly mediated by three distinct mechanisms. First, exosomes can activate intracellular signalling pathways through the interactions of exosome membrane proteins and receptors. For example, after the binding of TNF on the surface of dendritic cell-derived exosomes (DCexs) and its specific receptor on NK cells, the Based on the data in Table 2, latter could be stimulated to secrete interferon-γ (IFNγ) [87]. Secondly, exosomes can be actively transported along the cytoskeleton, actin filaments or microtubules in a rapid and directed pattern after internalization, which leads to signal transfer to targeted organelles such as lysosomes [88]. Thirdly, the exosome membrane can fuse with the target cell, leading to the release of its contents, including proteins, mRNAs and microRNAs, into the cytoplasm. Both the vesicle and the target cell express proteins and glycoproteins, by which they can complete exosome uptake [89]. Compared with traditional gene therapies, MSC-Exs, which are a type of nanocarrier, transfer specific molecules to recipients via

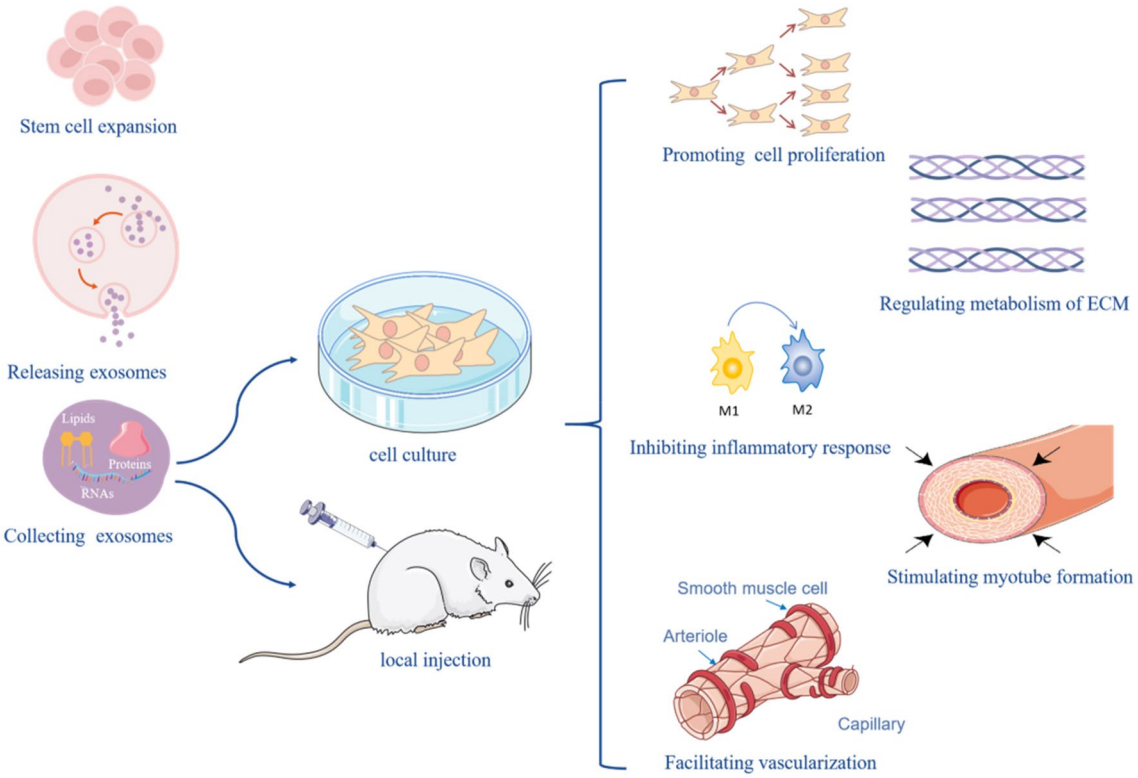

Fig. 2 Schematic presentation of the study design according to the references in Table 2

endocytosis and membrane fusion, achieving therapeutic effects with safety and precision [90, 91].

The role of MSC-Exs in PFD

Crucial changes in PFD pathology are deficiencies in fibroblast functions and ECM structure, OS in the pelvic floor and impaired muscle and nerves [41–43, 45]. MSC-Exs can stimulate the proliferation of fibroblasts, smooth muscle cells (SMCs) and Schwann cells and enhance fibroblast functions [106, 107]. Studies have shown that exosome therapy can reduce mesh exposure and promote hyperplasia in tissue and capillaries in mesh implantation sites in vivo [104, 108]. Exosomes tagged with the fluorescent dye PKH67 were internalized into target cells in vitro. Therefore, exosomes purified from MSCs may be a promising treatment for PFD via the consistent release of miRNAs and proteins to regulate targets (Table 2) [109].

Based on the data in Table 2, we can see various sources of MSCs that have been studied as cell-based therapies, and Fig. 2 shows the study designs of the references in Table 2. However, it is unclear which exosomes were more applicable and efficient. Given the concern regarding immunologic rejection, using autologous would be an ideal treatment option that could reverse the underlying pathologic condition. Adult MSCs are mainly isolated from adipose tissue, bone marrow or skeletal muscle, which can be more applicable than cells from other sources. Liposuction from the hip and thigh regions of the patient exemplifies an easy approach to ADSC isolation. The majority of MSC-Exs show powerful anti-inflammatory effects and promote proliferation-. However, we cannot determine which is the best choice for the treatment of PFD because each of the types has unique advantages and disadvantages. Moreover, there are apparent limitations in current studies: almost none of these studies used large animals. Finally, the effective exosome dose and injection sites vary in the current studies. As a result, the heterogeneity of the reviewed studies makes it difficult to draw firm conclusions.

Promoting cell proliferation

PFD patients exhibit impairments in function and decreased numbers of fibroblasts, myocytes, and nerve cells [110] in the pelvic floor environment [28]. The main cause of SUI is injuries to the pubococcygeal muscle, which is important for supporting pelvic organs [44, 111]. MSC-Exs exhibit obvious beneficial effects in rescuing these essential cells, which makes treatment based on MSC-Exs a viable therapeutic strategy [21, 112]. Increasing lines of evidence suggest a time- and dose-dependent increase in cell proliferation and migration and a decrease in apoptosis [21, 22, 113, 114],

which can restore cell and nerve fibre density and involves the TGFβ/SMAD [115], PI3K/AKT, Jak-STAT, Wnt, and Ras/ERK pathways. Ni indicated that local injection of exosomes promoted functional and histological recovery after SUI, which involved the PI3K-Akt, Jak-STAT, and Wnt pathways, as revealed by proteomic analysis [21]. Activation of the AKT pathway by MSC-Exs was related to a decrease in fibroblast apoptosis in vivo [107]. The PI3K/AKT/eNOS pathway mediates the biological events of MSC-Exs [116]. The Ras/ERK signalling pathway is crucial for the activation and proliferation of SCs, which is the first step in muscle regeneration and promotes the recovery of pubococcygeal muscle. MSC-Exs could enhance the phosphorylation of ERK1/2 and mediate significant improvements in urodynamic parameters and the function of injured muscle tissue [22, 117]. Wnt4 was contained in MSC-Exs, which promoted β-catenin nuclear translocation to enhance the proliferation and migration of cells in vivo, and this phenomenon could be reversed by the β-catenin inhibitor ICG001 or knockdown of Wnt4 in MSC-Exs in vitro [107]. Mechanistically, Wnt signalling targets the c-Myc gene, which regulates the cell cycle via the transition from G1 to S phase and shortens the cell cycle [118]. Similarly, Jagged 1, which is detected in MSC-Exs, is a ligand of the Notch pathway that shows the same effect on cell proliferation [119]. Zhao showed that MSC-Ex treatment could suppress cell apoptosis through another approach in vivo and in vitro, which inhibited nuclear translocation of apoptosis-inducing factor (AIF) and upregulated poly ADP-ribose polymerase 1 (PARP-1) and poly ADP-ribose (PAR), which play predominant roles in H_2O_2-induced cell death [120]. Furthermore, crosstalk between pathways could provide a foundation for MSC-Ex therapy. For example, Jagged1 (JAG1), the direct agonist of Notch, was recognized as an evolutionarily conserved target of the WNT/beta-catenin signalling pathway [121]. Wnt/β-catenin signalling was notably increased in response to the expression of JAG1 in vivo [118].

Regulating ECM metabolism

The major components of pelvic ECM, especially collagen I and III, constitute the supportive strength for pelvic organs. MMPs, especially MMP2 and MMP9, and TIMPs play fundamental roles in the balance of collagen synthesis and degradation, greatly contributing to ECM homeostasis [52]. However, severe disruption to this balance in the pelvic environment in PFD patients contributes to the weakness of pelvic connective tissues [5]. Luckily, accumulating evidence has suggested that MSC-Exs could enhance the supportive ability of pelvic connective tissues and obtain ideal therapeutic effects by overexpressing type I and III collagen and MMPs and downregulating TIMPs [106, 107, 122–125], resulting in increased collagen production [126] and ordered mature collagen [127]. Guo reported that the enhancement in ECM was achieved by activating TGF-β, which increased the phosphorylation level of SMAD and AKT [128]. However, MSC-Exs not only accelerate collagen production to repair ECM but also control these processes by delivering 14–3-3ζ to recruit YAP and p-LATS. As a result, the complex of YAP and p-LATS restricts excessive collagen deposition and subsequently inhibits Wnt/β-catenin signalling [129].

Inhibiting OS and the inflammatory response

An increase in OS activates the PARP pathway, which is responsible for regulating proinflammatory cytokine expression [60]. The involvement of inflammation and OS accelerates the disease process and has direct toxic effects on fibroblasts and Schwann cells [130]. MSC-Exs have been shown to play significant roles in the treatment of PFD because of their antioxidative effects against ROS and inflammation in fibroblasts [131, 132], which can be resolved by the induction of M1-M2 macrophage polarization [133]. There are several different mechanisms to explain this effect. In MSC-Ex, the level of the anti-inflammatory factor IL-10 was increased, while proinflammatory factors such as IL-1β, IL-8, IL-2 and IFN-γ were significantly decreased [134]. Liu reported that this effect was mediated by inhibiting the phosphorylation of AKT and overexpression of PTEN [135]. Another study showed that miR-223 in MSC-Exs played a fundamental role in the regulation of macrophage polarization by binding to homeobox domain PBX/Knotted 1 [136].

Taken together, these studies demonstrate the notable reparative function of MSC-Exs, such as improving the proliferation and activity of cells that are important for pelvic tissues and the release of inflammatory mediators via different signal pathways, suggesting that the administration of MSC-Exs is a potentially efficacious therapeutic strategy for PFD and could be of tremendous value in clinical settings.

Challenges in the use of MSC-Ex therapy for PFD

Despite the undeniable benefits of MSC-Exs in regeneration, great challenges still exist that impair their clinical application. It remains unclear which molecule in exosomes plays a key role in regulating the function of cells and the microenvironment in the pelvic floor. Therefore, more basic research should be conducted to explain the mechanism. At present, common methods in labs cannot meet these requirements, such as classical differential ultracentrifugation, commercial precipitation kits and physical separation approaches (e.g. ultrafiltration,

concentration and size-exclusion chromatography [137–139], and while differential ultracentrifugation is the gold standard for obtaining exosomes, with demands for operators and equipment, commercial precipitation kits can more easily obtain targets with smaller diameters; moreover, high costs and with the use of chemicals affect follow-up studies. Certain physical methods can avoid chemical contaminants; however, these methods may destroy exosome membrane structure and morphology, altering outcomes. Therefore, a suitable separation method should be developed to efficiently obtain purified exosomes, which is important for carrying out experiments and clinical applications. At present, local injection of exosomes is mainly used, but these particles rapidly dissipate. It is impossible to perform repeat injections in the clinic, which urges the search for better delivery approaches with good tissue receptivity, safety and ease of degeneration. Thus, to promote research on the application of exosomes, promising methods should be developed to produce exosomes on a large scale and establish efficient delivery systems and recognized administration protocols.

Future directions for MSC-Ex therapy in PFD

Currently, some methods are being developed to attain more exosomes with higher quality, which can help researchers obtain optimal results. It is acceptable and feasible to use technologies including preconditioning of MSCs, various types of biomaterial scaffolds and improved modification approaches to purify exosomes.

Preconditioning parental MSCs

Preconditioning, which enhances the function of exosomes, is a promising strategy to improve transplantation efficacy in vitro and in vivo [140]. Some studies have shown that pretreating or modifying parental MSCs can change exosome contents, including proteins and noncoding RNAs, which influences key downstream signalling pathways and improves treatment efficacy [25, 141, 142]. For example, it has been demonstrated that exosomes derived from MSCs infected by a lentivirus overexpressing TSG-6 can decrease the secretion of inflammatory molecules and ameliorate collagen deposition in vivo much more robustly than conventional exosomes [141]. Similarly, SIRT1-overexpressing human BMSCs showed improved effects via their exosomes by augmenting the proliferation, differentiation and activation of SCs via ERK signalling, resulting in the repair of urethral function in vivo [23]. The ability of MSC-Exs to augment the levels of growth factor expression and decrease the levels of proteins related to inflammation and OS can be increased by overexpressing nuclear factor-E2-related factor 2 (Nrf2), a transcription factor [132]. Interestingly, changes in the noncoding RNA levels in exosomes after preconditioning can greatly enhance their curative effects. MSCs preconditioned with lithium produce exosomes with more miR-1906, a new regulator of Toll-like receptor 4 (TLR4) signalling, which inhibits the NF-κB signalling pathway and enhances neuroprotective effects [113]. Coincidentally, other studies have shown that exosomes from MSCs that were pretreated with deferoxamine [142] or transfected with lncRNA H19 [143] were enriched in miR-126 and lncRNA H19, which contribute to ECM remodelling, proliferation, migration and the inhibition of inflammation [23].

Combination with tissue-engineered repair material

In addition to carrying out research on the contents, therapeutic mechanisms and effects of MSC-Exs, scholars have also focussed on safer and more effective delivery methods to replace injections. Tissue-engineered products are excellent candidates for delivery with outstanding biocompatibility, low cytotoxicity and immunogenicity and include but are not limited to hydrogels [144], decellularized scaffold ECM (dECM) [145] and combinations with other emerging technologies. These biological scaffolds have several advantages. First, these scaffolds can retain loaded exosomes, hydrogels can highly control exosomes by timed release for a long, continuous duration [109, 146], and the fibres of dECM can easily be combined with exosomes [109]. Furthermore, these materials can enhance therapeutic outcomes by providing a good environment in which cells obtain structure to activate biological events, such as adhesion, invasion, proliferation and the regeneration of neurons [109, 146, 147]. Surprisingly, the incorporation of MSC-Exs into hydrogels could effectively promote collagen deposition and remodelling and neuronal ingrowth [148].

Moreover, these delivery systems promote MSC secretion of more effective molecules (e.g. growth factors and cytokines) into exosomes to regulate MMP activity, the local inflammatory environment, and cell proliferation [109, 149]. It is also worth mentioning that these platforms can deliver other necessary cargoes in addition to exosomes. Yuan Xiong produced HA@MnO2/FGF-2/Exs that could provide rapid haemostasis and protection for wounds by concurrently releasing exosomes, oxygen and FGF-2 growth factor [150]. Thus, functional delivery approaches for exosomes offer emerging strategies for regenerative medicine, which can be applied to a variety of degenerative diseases [109].

Improving the yield of MSC-Exs

The quality and quantity of exosomes depend the status and number of MSCs. Future studies should determine the detailed environmental parameters (pH and temperature) for MSC culture to obtain effective and homogeneous exosomes. The inherent secretory ability of MSCs is impaired after several generations because of replicative senescence [151]. Some physical approaches have been shown to increase the yields of exosomes, such as the tangential flow filtration (TFF) system-based method [152] and ultrasonic shearing of MSCs for 1 min before conventional centrifugation and filtration [153]. Pretreatment of stem cells with lithium [113], cytochalasin B, and antifungal agents [154] can promote the production of exosomes.

Compared to conventional culture conditions, the hollow fibre 3D culture system was reported to enhance total exosome production up to 19.4-fold [155]. Moreover, the combination of 3D MSC cultures and TFF [156] or other biological materials can further promote the yields of exosomes. For example, 45S5 Bioglass® (BG), a well-known biomaterial, influences the formation and release of exosomes via the overexpression of neutral sphingomyelinase-2 (nSMase2) and Rab27a, which have been shown to activate the nSMase and Rab GTPase pathways, respectively [157].

Conclusion

PFD significantly compromises quality of life and confers great burdens on families and society. None of the current interventions provide satisfactory effects; therefore, studies are examining promising therapeutic approaches to escape this predicament. MSC-Exs have been reported to powerfully regulate various biological events associated with tissue regeneration, including cell proliferation, migration, ECM homeostasis and anti-inflammatory effects, and these methods are safe because of the lack of cell transplantation. Numerous findings have exciting therapeutic potential in PFD and other diseases. It is worth noting that these MSC-Exs could be tailored to maximize clinical effects through MSC preconditioning or certain delivery systems.

To date, the use of MSC-Exs is still in its infancy and has many limitations. Additional basic studies should be conducted to obtain a full understanding of the properties of exosomes, which include but are not limited to sources, biomarkers and biological functions. In addition, standardized treatment regimens and operating procedures should be established to ensure the effectiveness and safety of these interventions. Last but not least, larger reliable clinical studies will be required to validate previous findings and assess whether the balance between cost and benefits is reasonable.

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Declarations

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