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Review article

Innovating intervertebral disc degeneration therapy: Harnessing the power of extracellular vesicles

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ABSTRACT

Intervertebral disc degeneration is the leading cause of low back pain, imposing significant burdens on patients, societies, and economies. Advancements in regenerative medicine have spotlighted extracellular vesicles as promising nanoparticles for intervertebral disc degeneration treatment. Extracellular vesicles retain the potential of cell therapy and serve as carriers to deliver their cargo to target cells, thereby regulating cell activity. This review summarizes the biogenesis and molecular composition of extracellular vesicles and explores their therapeutic roles in intervertebral disc degeneration treatment through various mechanisms. These mechanisms include mitigating cell loss and senescence, delaying extracellular matrix degeneration, and modulating the inflammatory microenvironment. Additionally, it highlights recent efforts in engineering extracellular vesicles to enhance their targeting and therapeutic efficacy. The integration of extracellular vesicle-based acellular therapy is anticipated to drive significant advancements in disc regenerative medicine.

The translational potential of this article: Existing clinical treatment strategies often fail to effectively address the challenges associated with regenerating degenerated intervertebral discs. As a new regenerative medicine strategy, the extracellular vesicle strategy avoids the risks associated with cell transplantation and shows great promise in treating intervertebral disc degeneration by carrying therapeutic cargo. This review comprehensively examines the latest research, underlying mechanisms, and therapeutic potential of extracellular vesicles, offering a promising new strategy for intervertebral disc degeneration treatment.

1. Introduction

One of the most common musculoskeletal conditions in the world, low back pain (LBP) has a dramatic effect on patients' quality of life and places a heavy burden on societies and economies [1]. A comprehensive study covering 204 countries assessed the global incidence, prevalence, years lived with disability, and disability-adjusted life years for 371 diseases, finding that LBP is the main cause of nonfatal health loss globally [2]. Although there are several potential causes of LBP, intervertebral disc degeneration (IVDD) is a major contributing factor. About 40 % of long-term LBP cases are closely related to IVDD, a condition termed discogenic LBP [3]. As the world population ages, the number of people plagued by IVDD and discogenic LBP grows, making IVDD a prominent public health issue.

The intervertebral disc (IVD), a crucial component of the spinal functional unit, is a fibrocartilaginous tissue that connects neighboring vertebral bodies. It primarily consists of the central nucleus pulposus (NP), the surrounding annulus fibrosus (AF), and the cartilaginous endplates (CEP) situated on the upper and lower sides of the vertebrae. The IVD functions to cushion spinal loads and enhance spinal flexibility through its deformation. AF comprises layers of inter-lamellar shearing fibers and plays a role in transmitting pressure and resisting the lateral expansion of the IVD. The NP is a gel-like substance abundant in type II collagen and proteoglycans, playing a crucial role in buffering axial

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loads [4]. The CEP anchors the IVD and facilitates nutrition exchange between the neighboring vertebrae [5].

While the precise etiology of IVDD remains unclear, genetic predisposition, nutritional pathways, mechanical injury, lifestyle factors, and aging are all considered contributory factors [6]. These factors contribute to IVD cell death, extracellular matrix (ECM) degradation, pro-inflammatory cytokine release, biomechanical alterations, and osteochondral remodeling [7]. With aging, the CEP becomes thinner and eventually calcifies. These changes disrupt the homeostasis of the NP, impairing nutrient supply and waste removal, leading to a reduction in nucleus pulposus cell (NPC) numbers and an imbalance in ECM metabolism regulated by NPCs. Reduced proteoglycan and type II collagen expression within the ECM further induces increased NPC apoptosis and senescence, reducing NP hydration and resulting in decreased IVD height [8]. The decrease in NP hydration weakens its mechanical properties, placing more axial load on the surrounding AF. Structural biomechanical changes, combined with local chronic inflammation, lead to AF rupture. The ruptured AF allows for nerve ingrowth and neovascularization, which can cause chronic low back pain and limit patient mobility [9,10]. In the late stages of IVDD, the NP tissue might herniate through the ruptured AF into the spinal canal, causing spinal stenosis and nerve compression, leading to pain and functional impairment. Other late-stage outcomes include segmental spinal instability and spinal deformities, presenting as LBP, sciatica, and restricted mobility, severely impacting the patient's quality of life [11].

Currently, the primary clinical treatments for IVDD and related diseases are non-surgical and surgical treatment, both of which mainly focus on symptom management and delaying progression. As an early intervention, non-surgical treatments offer limited disease modification by reducing inflammation, alleviating pain, nourishing nerves, and relaxing muscles. Surgical strategies, like discectomy and spinal fusion, are essentially palliative and may fail to restore the baseline movement and mechanical bearing characteristics of the IVD [12]. Advanced regenerative strategies aim to address the underlying degenerative changes and maintain the homeostasis of the IVD and its microenvironment to achieve complete regeneration. Current clinical treatments have not yet met these ambitious goals [13]. In recent years, cell therapy, primarily involving stem cell treatments, has been proven to effectively slow down or even reverse the deteriorating microenvironment, promoting tissue repair and regeneration, and offering new therapeutic avenues for IVDD [14]. However, the application of stem cell therapy in discs faces challenges, including low cell survival rates [15], imprecise-induced differentiation [16], and tumor formation [17], which hinder the widespread adoption of stem cell transplantation. Extracellular vesicles (EVs), membrane-encapsulated particles, have been demonstrated in numerous studies to significantly contribute to the regenerative properties of cell therapy, making them a prospective IVDD therapy [18].

2. Cell therapy for IVDD

The regenerative mechanism of cell therapy involves delivering cells into the IVD, where they replenish lost cells through self-renewal or accelerate the healing process via paracrine signaling. This ultimately aims to restore the natural tissue morphology and biomechanical function of IVD [15]. The seed cells currently in use include resident cells and stem cells.

Most studies on IVDD focus on NPCs, which are the resident cells of IVD and are essential to preserving normal function [19]. Brissenden et al. designed a macroporous hydrogel formed from a triblock prepolymer through redox-initiated crosslinking for NPC delivery. In hydrogels prepared with the triblock copolymer but without chondroitin sulfate, NPCs were evenly distributed in clusters and deposited sulfated glycosaminoglycans (GAG) and type II collagen [20]. During IVD maturation and senescence, vacuolated notochordal cells (NCs) originating from the notochord are supplanted by chondrocyte-like NPCs. This transition coincides with the onset of IVDD, underscoring the pivotal role of NCs in disc homeostasis and health [21]. Vries et al. applied the NC-rich nucleus pulposus matrix (NCM) as a novel strategy for promoting the biological repair of IVDD. They found that bovine NPCs cultured on porcine NCM showed increased GAG content, achieved through increased proliferation of adolescent bovine NPCs and increased GAG production per adult bovine NPC [22]. However, the number of resident cells that can be isolated from the NP portion of the disc is limited, and there is a lack of healthy donor tissue sources. Additionally, primary cultured NP-derived somatic cells, such as NPCs and NCs, exhibit low cellularity and proliferation activity during the early stages, making large-scale in vitro expansion challenging, thus limiting their clinical application [23].

Induced pluripotent stem cells (iPSCs), generated from somatic cells through inducing ectopic expression of transcription factors, offer advantages such as easy sourcing, similarity to embryonic stem cells in function, and avoidance of ethical issues related to embryos [24]. Kamatani et al. demonstrated that implanting human induced pluripotent stem cell-derived cartilaginous tissue (hiPS-Cart) in nude rats could prevent IVDD and preserve the mechanical properties of the IVD. hiPS-Cart survived and populated the nuclectomized space after implantation into the disc, achieving spatial and functional replacement. scRNA-seq analysis demonstrated that the implanted hiPS-Cart cells were functional chondrocyte-like NPCs, indicating functional regeneration [25]. Nevertheless, the tumorigenicity of iPSCs remains a significant barrier to their research and clinical application. In contrast, mesenchymal stem cells (MSCs) have been reported to exhibit minimal tumorigenic potential while also demonstrating low immunogenicity [26]. MSC transplantation to the degenerated IVD not only replenishes depleted NPCs through differentiation but also improves the microenvironment via paracrine secretion of cytokines, chemokines, and anti-inflammatory molecules [27]. Researchers have transplanted MSCs into IVDs in various animal models, finding that MSCs replenished and even regenerated depleted NP and prevented the decline of type II collagen and proteoglycans [28,29].

Recent evidence suggests that the ability of stem cells to maintain the microenvironment homeostasis in IVD is primarily mediated through paracrine effects, with EVs playing a crucial role in these processes [30,31]. Compared to cell therapy, EVs offer a superior alternative characterized by non-proliferative nature, low immunogenicity, and ease of storage and transportation compared to cells. This approach overcomes the challenges associated with stem cell therapy, such as low survival rates, uncontrolled behavior, and imprecise-induced differentiation [32]. Numerous studies have demonstrated that EVs are instrumental in modulating the microenvironment within IVD, thereby promoting tissue regeneration and repair [30,33–35].

3. The biogenesis and components of EVs

EVs are membrane-encapsulated nanoparticles released into the extracellular milieu by different cell types. One notable characteristic of EVs is their capacity to preserve most of the function of their parent cells. Over the last two decades, EVs have garnered significant attention because of their unique characteristics, which include good stability, biocompatibility, safety, and capacity to pass various physiological barriers [36].

EVs may be categorized into several groups based on their origin, biogenesis, and release mechanisms: microvesicles, a.k.a. ectosomes (100–1000 nm in diameter), which originate from the plasma membrane, and exosomes (30–200 nm in diameter), which come from the endosomal system [37]. Additionally, some cellular processes also produce EVs, namely apoptotic bodies made during planned cell death and migrasomes generated during cell migration [38]. Given that exosomes are the most broadly investigated and used subtype of EVs, this review focuses on the biogenesis of exosomes. The early endosomes are formed by invagination of the plasma membrane and then merge to become late

endosomes. Subsequently, intraluminal vesicles (ILVs), are formed when the membranes of late endosomes invaginate into the lumen [39]. At this stage, the late endosomes, also referred to as multivesicular bodies containing ILVs, merge with the plasma membrane, secreting ILVs to the extracellular milieu as exosomes [40]. It should be noted that due to limitations in isolation techniques and the overlapping size ranges, distinguishing between exosomes and microvesicles becomes challenging. The exosomes or microvesicles sometimes represent a broader range of EVs [38].

As communication mediators of cell-to-cell, EVs can deliver diverse cellular molecules (such as nucleic acids, proteins, and lipids) and be absorbed by target cells to regulate metabolic imbalance, microenvironment, and cellular homeostasis, and alter the physiological morphology and function of target cells [16,41,42]. Furthermore, the content transported by EVs can reflect the pathologic state of the parent cells, involving different diseases and disease stages [43].

4. EV therapy for IVDD

These inherent properties endow EVs with potential applications in diagnosing and treating a range of diseases, involving IVDD. Recent studies have reported the relevant therapeutic roles of native EV cargo in IVDD, such as (1) inhibiting cell death; (2) delaying cell senescence; (3) maintaining ECM metabolic homeostasis; (4) modulating the inflammatory response and oxidative stress (Table 1) [44].

4.1. miRNAs

Among the therapeutic cargo of native EVs, miRNAs have been extensively studied and shown to play crucial roles. This review will delve into the mechanisms of miRNAs within EVs. miRNAs are non-coding single-stranded RNAs composed of 18–22 nucleotides that suppress target genes via pairing with their mRNAs and recruiting the

Table 1
The roles of extracellular vesicle cargos in intervertebral disc degeneration.

Cargo type	Cargos	Sources	Functions	Mechanism	Study types	References
miRNA	miR-21	BMSCs	Inhibits apoptosis of NPCs	Restrains PTEN and thus activates PI3K/Akt pathway	In vitro and in vivo	[45]
	miR-142-3p	BMSCs	Ameliorates apoptosis of NPCs	Suppresses MAPK signaling by targeting MLK3	In vitro	[46]
	miR-31-5p	MSCs	Inhibits apoptosis and calcification in EPCs	Negatively regulates ATF6-related endoplasmic reticulum stress	In vitro and in vivo	[47]
	miR-21-5p	BMSCs	Promotes proliferation of AF cells	Post-transcriptionally regulates PTEN	In vitro	[48]
	miR-217	BMSCs	Prevents NPC apoptosis and ECM degradation by promoting autophagy	Targets EZH2, and consequently upregulates FOXO3 expression	In vitro and in vivo	[49]
	miR-125-5p	CESCs	Promotes NPC autophagy and inhibits apoptosis and ECM degradation	Targets SUV39H1	In vitro and in vivo	[50]
	miR-155	BMSCs	Activates autophagy and inhibits the apoptosis level of NPCs	Targets Bach1 and in turn increases HO-1 protein expression	In vitro	[51]
	miR-155-5p	ASCs	Promotes autophagy and inhibits pyroptosis in NPCs	Targets TGFβR2	In vitro and in vivo	[52]
	miR-3594-5p	BMSCs	Relieves senescence in NPSCs	Targets HIPK2	In vitro and in vivo	[53]
	miR-105-5p	iMSCs	Relieves the senescence of NPCs	Downregulates PDE4D expression and activates the Sirt6 signaling pathway	In vitro and in vivo	[54]
	miR-27a	NPCs	Suppresses ECM degradation in NPCs	Targets MMP-13	In vitro	[55]
	miR-31	BMSC	Promotes NPC proliferation and reduces cell apoptosis and ECM degradation	Inhibits NFAT5 expression, leading to activation of the Wnt/β-catenin pathway	In vitro and in vivo	[56]
	miR-410	MSCs	Inhibits NPCs pyroptosis	Targets NLRP3	In vitro and in vivo	[57]
	miR-302c	ESCs	Inhibits NPC pyroptosis	Targets NLRP3	In vitro and in vivo	[58]
	miR-26a-5p	ucMSCs	Inhibits NPC pyroptosis	Targets METTL14 and downregulates NLRP3	In vitro	[59]
circRNA	circ_0072464	BMSCs	Inhibits NPC ferroptosis	Upregulates of miR-431-mediated NRF2	In vitro and in vivo	[60]
	circ_0050205	BMSCs	Promotes NPC survival and inhibits ECM degradation	Upregulates of miR-665-mediated GPX4	In vitro and in vivo	[61]
lncRNA	CAHM	BMSCs	Reduces apoptosis and ECM degradation of NPCs	Inhibits M1-type macrophage polarization	In vitro and in vivo	[62]
	MALAT1	Platelet-rich plasma	Downregulates NPC pyroptosis and ECM degradation	Binds to miR-217 to promote SIRT1 expression and then blocks the NF-κB/NLRP3 pathway	In vitro and in vivo	[63]
Protein	SIRT1	Platelet-rich plasma	Restores mitochondrial function in NPCs	Activates the PGC1α–TFAM pathway	In vitro and in vivo	[64]
	Vasorin	BMSCs	Promotes the proliferation and ECM anabolism of NPCs	Activates the Notch1 signaling pathway	In vitro and in vivo	[65]
	MATN3	USCs	Promotes NPC proliferation and ECM Synthesis	Activates TGF-β, which elevates the phosphorylation level of SMAD and AKT	In vitro and in vivo	[66]
	NAMPT	Adipocytes	Rejuvenates the senescence of NPCs and EPCs	Activates NAD^+ biosynthesis and Sirt1 activity	In vitro and in vivo	[67]

BMSCs: Bone marrow mesenchymal stem cells; NPCs: Nucleus pulposus cells; PTEN: Phosphatase and tensin homolog; PI3K: Phosphatidylinositol-3-kinase; MAPK: Mitogen-activated protein kinase; MLK3: Mixed lineage kinase 3; MSCs: Mesenchymal stem cells; EPCs: Endplate chondrocytes; ATF6: Activating transcription factor 6; AF: Annulus fibrosus; ECM: Extracellular matrix; EZH2: Enhancer of zeste homolog 2; FOXO3: Forkhead box O-3; CESCs: Cartilage endplate stem cells; SUV39H1: Suppressor of variegation 3–9 homolog 1; Bach1: BTB and CNC homology 1; HO-1: Heme Oxygenase-1; ASCs: Adipose tissue stem cells; TGFβR2: Transforming growth factor-β receptor 2; NPSCs: Nucleus pulposus stem cells; HIPK2: Homeodomain-interacting protein kinase 2; iMSCs: Induced pluripotent stem cell-derived mesenchymal stem cells; PDE4D: Pharmacological phosphodiesterase 4D; Sirt6: Sirtuin 6; MMP-13: Matrix metalloproteinase-13; NFAT5: nuclear factor of activated T cells 5; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; ucMSCs: umbilical cord mesenchymal stem cells; METTL14: Methyltransferase-like 14; NRF2: Nuclear factor erythroid 2-related factor 2; GPX4: Glutathione Peroxidase 4; CAHM: Colorectal adenocarcinoma hypermethylated; MALAT1: Metastasis associated lung adenocarcinoma transcript 1; SIRT1: Sirtuin1; PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1α; TFAM: Mitochondrial transcription factor A; MATN3: Matrilin-3; USCs: Urine-derived stem cells; TGF-β: Transforming growth factor-β; NAMPT: Nicotinamide phosphoribosyltransferase.

miRNA-induced silencing complex (miRISC) [68,69].

Alleviating cell death, particularly the apoptosis of NPCs, is regarded as an effective therapeutic strategy (Fig. 1a). According to Cheng et al., miR-21 has anti-apoptotic and anti-IVDD properties. It is downregulated in apoptotic NPCs and enriched in exosomes derived from MSCs. Subsequent in vitro and in vivo research confirmed that the exosomal miR-21 activates the phosphatidylinositol 3-kinase (PI3K)-Akt pathway associated with survival signaling by targeting phosphatase and tensin homolog (PTEN), ultimately alleviating NPC apoptosis and IVDD [45]. Similarly, Zhu et al. revealed that exosomes generated from MSCs carry miR-142-3p and negatively regulate the mitogen-activated protein kinase (MAPK) signaling pathway by targeting Mixed Lineage Kinase 3 (MLK3) in NPCs. Inhibition of MAPK activity has protective effects against NPC apoptosis and inflammatory response induced by IL-1β, alleviating the progression of IVDD [46].

CEP degeneration accelerates the progression of IVDD by altering mechanical loading, upregulating the levels of inflammatory cytokines in NPCs, and increasing apoptosis [34,70]. Xie et al. found that MSC-derived exosomes alleviate the apoptosis and calcification of endplate chondrocytes (EPCs) induced by tert-butyl hydroperoxide (TBHP). These protective effects are attributed to exosomal miR-31-5p, which targets Activating Transcription Factor 6 (ATF6) and negatively regulates ATF6-related endoplasmic reticulum stress [47]. Zhou et al. identified miR-31-5p as a crucial regulatory factor in IVDD, with its levels downregulated in this condition. miR-31-5p overexpression inhibits NPC apoptosis, promotes proliferation, enhances ECM synthesis, and suppresses matrix-degrading enzymes in NPCs by modulating the stromal cell-derived factor 1 (SDF-1)/chemokine receptor 4 (CXCR7) axis [71].

Continued progression of IVDD is associated with a loss of AF integrity. A study has shown that exosomes from bone marrow-derived mesenchymal stem cells (BMSCs) inhibit autophagy of AF cells and thus mitigate inflammation and apoptosis induced by IL-1β, although the active components within these exosomes remain unidentified [72]. DiStefano et al. observed that exosomes derived from MSCs improve AF cell proliferation by post-transcriptional regulation and shield them from IVDD-associated abnormal phenotypes. RNA-seq results indicated that MSC-derived exosomes alleviate IVDD by delivering miR-21-5p, which regulates cellular division. Additionally, the decrease of miR-214-5p and miR-652-3p might be linked to increased AF cell motility [48].

Autophagy is a vital catabolic process that aids in cellular quality control under the harsh microenvironment by selectively removing and recycling damaged, senescent, or excess biological macromolecules and organelles [34]. Hao et al. demonstrated that by delivering miR-217, EVs derived from MSCs inhibit NPC apoptosis and ECM degradation. They found that the enhancer of zeste homolog 2 (EZH2) is a target of miR-217 in this process, leading to a decrease in EZH2 levels. The reduced expression of EZH2 disrupts its binding to the forkhead box O-3 (FOXO3) promoter, thereby increasing the expression of FOXO3. This increased FOXO3 stimulates autophagy. Consequently, miR-217 in EVs alleviates IVDD by interfering with EZH2-mediated repression of FOXO3, hindering NPC apoptosis and ECM degradation [49]. Another study discovered that cartilage endplate stem cells (CESCs)-derived exosomes transfer miR-125-5p to NPCs and facilitate NPC autophagy, reduce apoptosis, and mitigate ECM degeneration by targeting the suppressor of variegation 3–9 homolog 1 (SUV39H1) gene [50]. Similarly, BMSC-derived exosomes deliver miR-155, which restrains the BTB and CNC homology 1 (Bach1) and in turn, increases Heme Oxygenase-1 (HO-1) expression, thereby promoting NPC autophagy and reducing apoptosis in NPCs, thus alleviating IVDD [51]. Coincidentally, another study isolated exosomes containing miR-155-5p from human adipose tissue stem cells. This miRNA targets transforming growth factor-β receptor 2 (TGFβR2), promoting NPC autophagy and inhibiting cell pyroptosis [52].

Senescent IVD cells exhibit irreversible growth arrest under various stresses and affect the microenvironment of adjacent cells and tissues through the secretion of the senescence-associated secretory phenotype (SASP) (Fig. 1b) [73]. Peng et al. developed an arginine-glycine-aspartic acid tripeptide (RGD)-complexed NP matrix hydrogel capable of effectively anchoring small EVs, thereby enhancing the retention and bioavailability of EVs to promote IVD regeneration. Small RNA

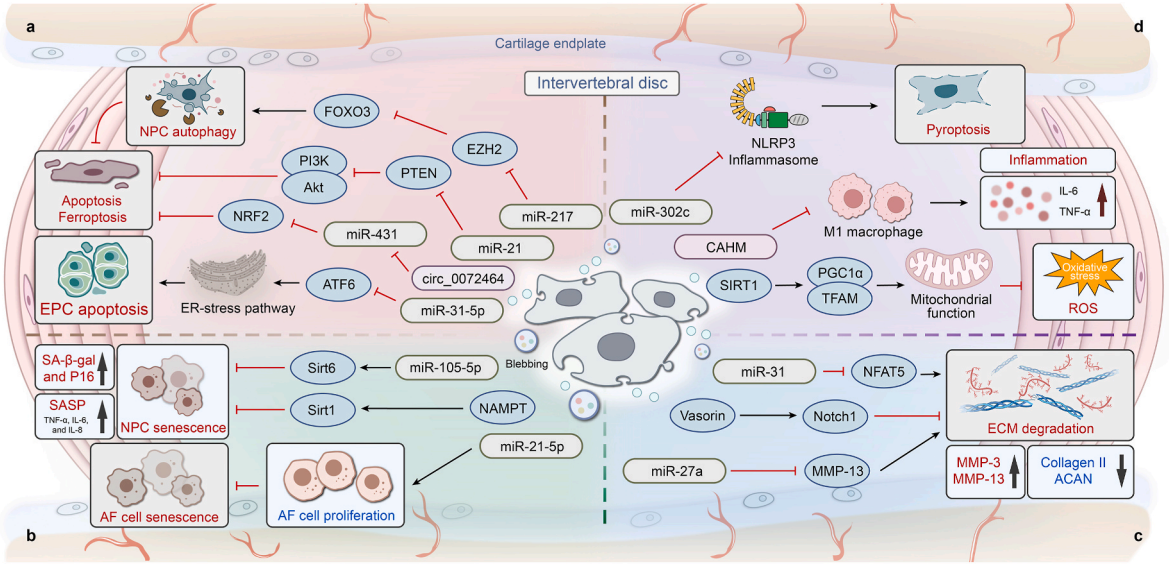

Fig. 1. Illustration of the functions and mechanisms that native extracellular vesicles (EVs) exert in intervertebral disc degeneration. EVs deliver a variety of cellular molecules, such as nucleic acids and proteins, to intervertebral disc cells, enhancing their capacity to respond to the diverse pathological changes associated with disc degeneration. a. Through mechanisms such as promoting autophagy and inhibiting endoplasmic reticulum (ER) stress pathways, EVs prevent programmed cell death in disc cells. b. EVs can delay or even reverse disc cell senescence by increasing the expression of sirtuin (Sirt) 1 and 6, or by promoting cell proliferation. c. Furthermore, EVs mitigate extracellular matrix (ECM) degeneration characterized by elevated matrix metalloproteinases (MMPs) and decreased levels of type II collagen and aggrecan (ACAN). d. EVs also suppress NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasomes, modulate macrophage phenotypes, and restore mitochondrial function, thus reducing inflammation-induced pyroptosis and lowering levels of inflammatory cytokines and reactive oxygen species (ROS).

sequencing and miRanda analysis identified that miR-3594-5p is highly expressed in these EVs and targets homeodomain-interacting protein kinase 2 (HIPK2), which is associated with cellular senescence [53]. MSCs derived from iPSCs (iMSCs) offer the advantage of being readily accessible and rapidly proliferative, facilitating the large-scale production of EVs [74]. Sun et al. discovered that injecting iMSC-derived small EVs (iMSC-sEVs) into the IVD mitigates the progression of NPC senescence. These EVs were shown to deliver miR-105-5p, leading to a reduction in the expression of cAMP-specific hydrolase phosphodiesterase 4D (PDE4D). This reduction subsequently activates the Sirtuin 6 (Sirt6) pathway, which is essential for DNA repair and promoting longevity [54].

The structure and function of ECM are largely controlled by metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) (Fig. 1c) [75]. A recent study demonstrated that miR-27a delivered by exosomes from autophagy-activated NPCs can target MMP-13 in NPCs thereby mitigating IL-1β-induced ECM degradation, ultimately alleviating IVDD [55]. Several studies have demonstrated that EVs can transfer other miRNAs, like miR-217, miR-125-5p, and miR-31, to target cells increasing type II collagen and aggrecan levels while lowering MMP or ADAMTS levels, thus delaying ECM deterioration [49,50,56].

The increase in inflammatory cells and cytokines triggers inflammatory cascades and various signaling pathways, leading to IVD cell death and ECM degradation (Fig. 1d) [76,77]. Zhang et al. found that pyroptosis of NPCs mediated by NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) is activated in IVDD models while MSCs treatment can reverse this process. Furthermore, they revealed that MSCs-derived exosomes can deliver miR-410, which directly targets NLRP3 to exert anti-pyroptotic effects on NPCs [57]. Yu et al. discovered that exosomal miR-302c also inhibits NLRP3, thereby mitigating pyroptosis in NPCs [58]. Similarly, exosomes from human umbilical cord MSCs, carrying miR-26a-5p, reduce the expression of methyltransferase-like 14 (METTL14) in NPCs. The loss of METTL14 reduces NLRP3 inflammasome levels, leading to decreased pyroptosis and pro-inflammatory cytokines [59].

4.2. circRNAs

Circular RNAs (circRNAs), featuring a covalently closed circular loop structure, effectively sponge miRNAs, thereby releasing their target mRNAs [78]. Yu et al. discovered that BMSC-derived EVs could alleviate IVDD in mice, potentially due to the presence of circ_0072464. Further experiments showed that circ_0072464 sponges miR-431, upregulating its target, nuclear factor erythroid 2-related factor 2 (NRF2), eventually inhibiting NPC ferroptosis and enhancing the ability of EVs to promote NPC proliferation and matrix synthesis [60]. Similarly, BMSC-derived EVs deliver circ_0050205 to NPCs, which inhibits miR-665, thereby upregulating Glutathione Peroxidase 4 (GPX4) expression, increasing NPC survival, and reducing ECM degradation [61]. Additionally, it has been demonstrated that some circRNAs carry out their biological functions by regulating gene expression at both the transcription and post-transcriptional levels [79]. This may suggest another potential mechanism for EV-derived circRNA therapy in treating IVDD.

4.3. lncRNAs

Long non-coding RNAs (lncRNAs) are defined as a diverse class of non-coding RNAs with more than 200 nucleotides. They regulate crucial cellular physiological processes, including DNA methylation, chromatin modification, transcription, post-transcriptional modifications, and translation [80]. lncRNAs, like miRNAs and other short non-coding RNAs, have a role in IVDD. Li et al. isolated exosomes from BMSC culture media and injected them into the degenerated IVDs of rats. They found that the exosomes inhibited Pro-inflammatory M1 macrophage polarization and inflammation, reduced NPC apoptosis, and mitigated ECM degradation. This effect was reversed upon knockout of the lncRNA colorectal adenocarcinoma hypermethylated (CAHM), suggesting that exosomes ameliorate IVDD by delivering CAHM, which suppresses M1 macrophage polarization and ECM degradation [62]. Tao et al. discovered that platelet-rich plasma-derived extracellular vesicles (PRP-EVs) could reverse tert-butyl hydroperoxide-induced NPC pyroptosis and ECM degradation. PRP-EVs upregulated the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) expression in NPCs, and knockdown of MALAT1 exacerbated cell damage. Further experiments demonstrated that MALAT1 delivered to NPCs increases the expression of Sirtuin1(SIRT1) by downregulating miR-217, ultimately alleviating NLRP3 inflammasome activation and NPC pyroptosis [63].

4.4. Protein

EV proteome is distributed over numerous critical biochemical and cellular processes which are essential for EV biogenesis, secretion, cell structure, cell communication, microenvironment, metabolism, and tissue repair and regeneration [81].

Xia et al. found that exosome uptake by NPCs protected mitochondrial structure and function. Proteomic analysis revealed that 10.7 % of the exosomal proteins were mitochondrial-related, suggesting that exosomes might mitigate mitochondrial degradation by supplementing several critical mitochondrial proteins [82]. Dai et al. identified the presence of the mitochondria-associated protein SIRT1 in platelet-derived extracellular vesicles (PEVs) through proteomic analysis. Through the activation of the SIRT1–peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α)–mitochondrial transcription factor A (TFAM) pathway, PEVs restore impaired NPC mitochondrial function. Subsequent in vivo experiments also demonstrated that PEVs lower the level of reactive oxygen species (ROS) in mitochondria and restore mitochondrial function, thereby delaying IVDD progression [64].

In addition to mitochondrial-related proteins, EVs can deliver functional proteins to reduce NPC apoptosis/pyroptosis or rejuvenate senescent NPCs. Liao et al. revealed that EVs containing vasorin promote NPC proliferation and ECM anabolic processes via the activation of the Notch1 signaling pathway, thereby benefiting IVDD repair [65]. Guo et al. found that compared to normal NP tissue, matrilin-3 (MATN3) significantly decreases in degenerated NP tissue, whereas urine-derived stem cell exosomes (USC-Exos) are rich in MATN3 protein. MATN3 in USC-Exos promotes NPC proliferation and ECM synthesis in IVDD rats. Furthermore, they elucidated that MATN3 exerts its effects by activating transforming growth factor-β (TGF-β), subsequently upregulating the phosphorylation level of SMAD and AKT [66]. In mammalian NAD^+ biosynthesis, nicotinamide phosphoribosyltransferase (NAMPT) is a rate-limiting enzyme, that acts as a controller of the intracellular NAD^+ pool [83]. Sun et al. found that adipocyte-derived small EVs have therapeutic effects on the senescence of NPCs and EPCs in vitro. Moreover, they observed that small EVs carrying NAMPT stimulate NAD^+ biosynthesis and the anti-senescence-related Sirt1 pathway, ultimately restoring senescent NPCs and EPCs [67].

5. Engineered EVs for IVDD

EVs possess high in vivo circulatory stability, lack toxicity, have low immunogenicity, and exhibit good biocompatibility [84]. However, native EVs face several challenges, including insufficient tissue targeting, rapid clearance, short-lived effects, and limited uptake by target cells. These issues significantly hinder the clinical application of EVs [32,85]. Additionally, due to the limited blood flow, low nutrient levels, and acidic environment of the disc, the targeting ability of EVs is further diminished. To enhance the targeted delivery efficiency and therapeutic efficacy of EVs in the treatment of IVDD, engineering techniques (Fig. 2) have been utilized to optimize EVs [86]. There are two primary

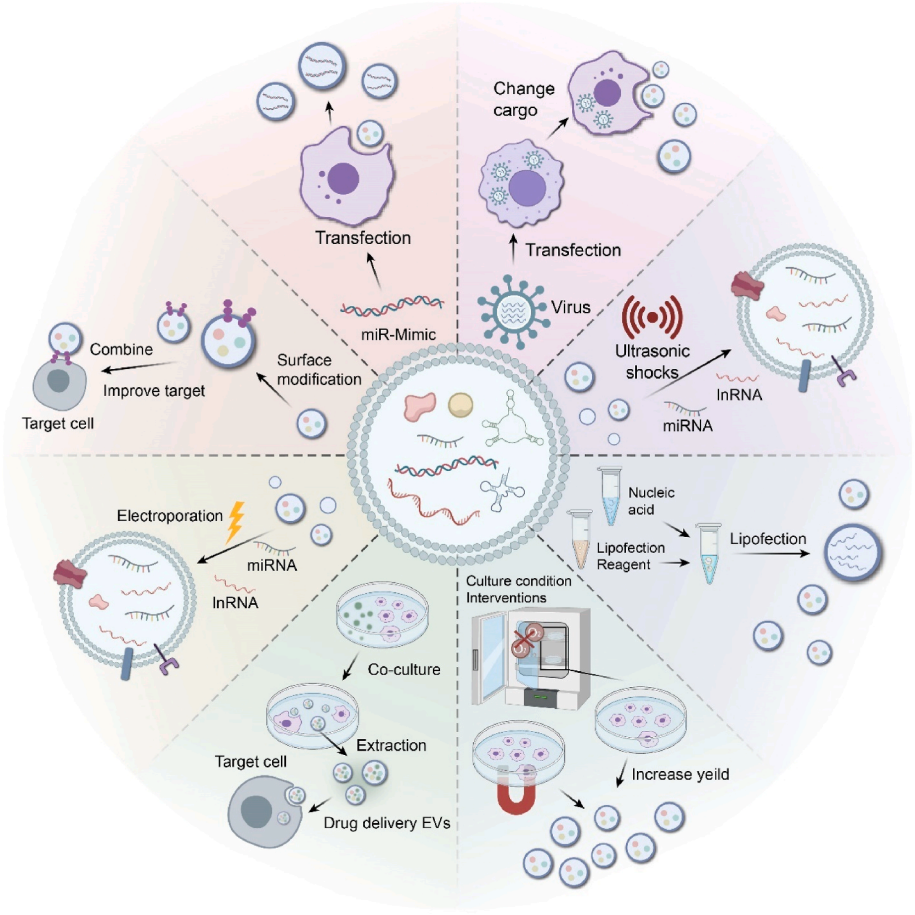

Fig. 2. Engineering strategies of extracellular vesicles (EVs) applied to intervertebral disc degeneration (IVDD). Engineering strategies have endowed EVs with enhanced targeting capabilities and more pronounced therapeutic effects. Current methods for EV engineering in the context of IVDD can be broadly categorized into indirect and direct approaches. Indirect engineering methods include utilizing gene editing technology to alter surface proteins and encapsulated cargo, co-culturing with parent cells to load therapeutic agents, and culturing parent cells under specific conditions to improve EV yield. Direct engineering techniques involve the immediate loading of therapeutic molecules into EVs through methods such as sonication, electroporation, and lipofection.

approaches for the engineered modification of native EVs: indirect EV engineering and direct EV engineering (Table 2).

5.1. *Indirect EV engineering*

Studies have shown that the content of secreted EVs is influenced by the parent cells [87]. For instance, parent cells can quickly react to the extracellular environment and regulate EV production and bioactivity, which regulates the immune response and homeostasis [88]. Therefore, various cellular-level approaches, such as gene transfection, modification of the culture environment, and chemical induction, have been applied to increase EV production or enhance tissue regeneration.

5.1.1. *Surface modification through parent cells*

Proteomics studies have revealed that various proteins exist in the membranes of EVs [89], many of which are involved in surface functionalization for targeting, signal transduction, and cell activation. Providing targeting moieties to EVs to either selectively enrich them in specific cells or inhibit their absorption by non-target cells is one of the primary objectives of surface engineering [90]. For instance, Liao et al. showed that EVs derived from MSCs exert anti-pyroptosis effects in NPCs by delivering antioxidant proteins such as peroxiredoxin-2 (Prx-2). Moreover, caveolae-associated protein 2 (Cavin-2) on the EV membrane enhances NPC absorption of EVs, thereby increasing their protective efficacy. Therefore, researchers transfected MSCs with Cavin-2 expression vectors to produce engineered EVs expressing Cavin-2 on their membranes. These engineered EVs stably express Cavin-2 on their membranes, restoring the uptake rate of EVs in tumor necrosis factor-α (TNF-α)-treated NPCs, effectively improving cell survival and delaying IVDD progression in vitro [91].

5.1.2. *Loading cargo through parent cells*

As previously discussed, the unique structure of EVs, which comprises a hydrophilic core and a hydrophobic phospholipid bilayer, enables the introduction of various cargos. In terms of protein transfection, Luo et al. transfected CESCs with lentivirus carrying the sphingosine kinase 2 (Sphk2) gene, enabling them to secrete exosomes with higher levels of Sphk2 protein. The study demonstrated that engineered exosomes carrying Sphk2 entered NPCs, improving autophagy by activating the Sphk2/PI3K/AKT pathway, thereby inhibiting cellular senescence. Subsequently, they constructed hydrogels modified with the extracellular matrix of costal cartilage containing gene-edited CESCs to promote cell growth, sustain exosome release, and ultimately inhibit the progression of IVDD [92]. Regarding RNA transfection, two strategies are currently employed for miRNA transfection into EVs: using double-stranded miRNA mimics to confer or enhance miRNA function in EVs, and using antimiR oligonucleotides to inhibit miRNA function in EVs [93]. miRNA mimics serve as the core of miRNA replacement therapy, providing activity identical to endogenous miRNA [94]. For example, Cui et al. transfected miRNA-129-5p mimic into BMSCs to obtain EVs carrying miR-129-5p. These EVs suppressed NPC apoptosis, ECM degradation, and macrophage M1 polarization by delivering

Table 2
Application of engineered extracellular vesicles for intervertebral disc degeneration.

Engineering strategy	Function	Active ingredients	Mechanism	Study types	References
Surface modification through parent cells	Protect against pyroptosis of NPCs	Prx-2; Cavin-2	Regulates inflammasome activation; Restores the EVs Uptake of NPCs	In vitro	[91]
Loading cargo through parent Cells	Activate autophagy and inhibit the senescence of NPCs	Sphk2	Activates the PI3K/AKT pathway	In vitro and in vivo	[92]
	Decrease NPC apoptosis, ECM degradation, and M1 polarization of macrophages	miR-129-5p	Targets LRG1 and inactivates the p38 MAPK signaling pathway	In vitro and in vivo	[95]
	Promote chondrogenic differentiation of NP-MSCs	miR-15a	Downregulates MMP-3 through PI3K/Akt and Wnt3a/β-catenin axis	In vitro	[96]
	Attenuate apoptosis and inflammation and elevate cell migration and proliferation of NPCs	AntagomiR-4450	Inhibits miR-4450 to upregulate ZNF121	In vitro and in vivo	[98]
Culture condition interventions for parent cells	Rejuvenate NPC senescence and restore ECM deposition	GLRX3	Modulates the redox homeostasis	In vitro and in vivo	[102]
	Alleviate the inflammation, promote NPC proliferation, and enhance proteoglycan synthesis and collagen formation	miR-7-5p	Suppresses the NF-κB/Cxcl2 axis	In vitro and in vivo	[103]
	Promote the NPCs proliferation and synthesis of ECM	miR-17-5p	Suppresses TLR4 signaling pathway	In vitro and in vivo	[104]
	Inhibit angiogenesis	miR-140-5p	Downregulates Wnt11 expression and inhibits β-catenin nuclear accumulation	In vitro and in vivo	[106]
	Attenuate NPC senescence	Mitochondria	Upregulated the expression of Kif5b and its interaction with Rab22a	In vitro and in vivo	[108]
Drug inducement for parent cells	Suppress ECM degradation	miR-27a	Restrains MMP-13	In vitro	[55]
	Rejuvenate NPCs senescence	ITIH4	Activates the Akt signaling	In vitro and in vivo	[112]
	Inhibit the apoptotic rates, ECM degradation, and fibrosis deposition in NPCs	miR-532-5p	Targets RASSF5	In vitro	[113]
Electroporation	Reprogram NPCs into a healthier anti-catabolic and anti-inflammatory state	FOXF1 mRNA	Increases GAG accumulation, and decreases inflammatory, catabolic, and pain-associated factors	In vitro and in vivo	[119]
Lipofection	Diminish NPC senescence	ATR	Inhibits cGAS-STING axis-dependent pathway	In vitro and in vivo	[121]
Sonication	Protect AF	miR-378	Promotes AF cell proliferation and migration, inhibits pathological ECM remodeling, and restores the mitophagy of AF cells	In vitro and in vivo	[123]

NPCs: Nucleus pulposus cells; Prx-2: Peroxiredoxin-2; Cavin-2: Caveolae-associated protein 2; EVs: Extracellular vesicles; Sphk2: Sphingosine kinase 2; PI3K: Phosphatidylinositol 3-kinase; ECM: Extracellular matrix; LRG1: Leucine-rich α2-glycoprotein1; MAPK: Mitogen-activated protein kinase; NP-MSCs: Nucleus pulposus -mesenchymal stem cells; MMP: Matrix metalloproteinase; ZNF121: Zinc finger protein-121; GLRX3: Glutaredoxin 3; TLR4: Toll-like receptor 4; Kif5b: Kinesin family member 5B; ITIH4: Inter-α-trypsin inhibitor heavy chain H4; RASSF5: Ras association domain family member 5; FOXF1: Forkhead-box F1; GAG: Glycosaminoglycans; ATR: Ataxia-telangiectasia-mutated and Rad3-related protein; cGAS: Cyclic GMP-AMP synthase; STING: Stimulator of interferon genes; AF: Annulus fibrosus.

miR-129-5p. Further mechanistic studies revealed that miR-129-5p exerted these effects by targeting leucine-rich α2-glycoprotein1 (LRG1) and disrupting the p38 MAPK pathway in NPCs, thereby mitigating IVDD progression [95]. Additionally, Zhang et al. constructed NPCs transfected with miR-15a mimic secreted exosomes with high levels of miR-15a (exo-miR-15a). Exo-miR-15a positively regulated the NP-MSCs proliferation and colony formation by decreasing the phosphorylation of PI3K/Akt and upregulating Wnt3/β-catenin [96]. Antagomirs are chemically modified miRNA inhibitors and can competitively bind to mature miRNAs in vivo, making them useful for loss-of-function studies [97]. Yuan et al. employed a miRNA lentiviral vector system to produce placental MSC-derived exosomes capable of delivering AntagomiR-4450 to alleviate IVDD and improve gait abnormality. Delivery of AntagomiR-4450 to NPCs inhibited miR-4450's targeting of zinc finger protein-121 (ZNF121), restoring ZNF121's protective effects on NPCs [98].

5.1.3. Culture condition interventions for parent cells

In the avascular and hypoxic environment of the NP, NPCs can survive by expressing hypoxia-inducible factor (HIF) −1 and −2, which activate the expression of the downstream gene to improve the supply and decrease consumption of oxygen [99]. Similarly, hypoxic preconditioning of MSCs has been demonstrated as an effective engineering strategy to enhance their survival, proliferation, migration, differentiation, ECM deposition, and the therapeutic efficacy of their secreted EVs [100,101]. Liu et al. pretreated MSCs with hypoxia to obtain EVs enriched with Glutaredoxin 3 (GLRX3). GLRX3 carried by these EVs significantly enhanced the antioxidative defenses of NPCs, thereby preventing the accumulation of ROS and the subsequent cascade of cellular senescence. Following this, they designed a biopolymer-based supramolecular hydrogel to deliver EVs-GLRX3, which exhibited properties of injectability, biodegradability, and ROS responsiveness [102]. In addition to protein cargo, hypoxic preconditioning has been demonstrated to promote the expression of specific miRNAs in MSC-derived EVs. Another study found a significant increase of miR-7-5p in EVs from hypoxia-protreated MSCs. These EVs promoted anti-inflammatory responses, NPC proliferation, proteoglycan synthesis, and collagen formation through the miR-7-5p/NF-κB/Cxcl2 axis, highlighting their promise as a therapeutic strategy for IVDD and LBP [103]. Zhou et al. discovered that hypoxia-preconditioned MSCs secreted small

EVs with high levels of miR-17-5p. EVs miR-17-5p facilitated NPC proliferation and ECM synthesis by inhibiting the Toll-like receptor 4 (TLR4) pathway, thereby exerting therapeutic effects on IVDD [104].

Similar to hypoxic conditions, physical stimuli can also affect the biogenesis, secretion, and cargo of EVs. Mechanical stress is a constant and dynamic environmental factor influencing metabolic processes, apoptosis, and senescence within the IVD. To imitate the mechanical conditions experienced by the NP during normal activities, Liu et al. constructed and tested a compression device capable of applying periodic mechanical stress (PMS). They found that PMS effectively reduced osteogenic differentiation and promoted the synthesis of ECM proteins, thereby enhancing structural NP recovery [105]. In the field of EVs, Sun et al. reported that compressive load cultures of NCs resulted in an increased concentration of NC-derived exosomes. These exosomes, secreted under compressive load, delivered high levels of miR-140-5p into endothelial cells and inhibited the invasion of blood vessels by targeting the Wnt/β-catenin pathway, thus exhibiting potential therapeutic effects for IVDD [106].

Beyond mechanical stress, other forms of physical stimuli, such as static magnetic fields, also serve as effective means for the indirect engineering of EVs. A previous study revealed that magnetic fields can directly stimulate and enhance the secretion of microvesicles from equine adipose-derived MSCs, with the secreted microvesicles being enriched in specific growth factors compared to controls [107]. Shi and colleagues demonstrated that MSCs can delay the senescence of NPCs by secreting mitochondria-containing microvesicles (mitoMVs). Furthermore, stimulation with static magnetic fields was found to enhance mitoMV secretion by promoting cargo transport and plasma membrane budding. They further uncovered that static magnetic field stimulation significantly upregulated the expression of kinesin family member 5B (Kif5b), a protein related to mitochondrial transport, and its interaction with the membrane-associated GTPase Rab22a. By constructing a gelatin methacrylate (GelMA) hydrogel delivery system enriched with mitoMVs, they effectively achieved the engineered MVs' function of delaying IVDD in vivo [108].

Other physical stimuli such as light, ultrasound, and dehydration have also been reported to affect the biological processes of parent cells, thereby influencing the biogenesis and function of EVs [109–111]. However, these stimuli have not yet been applied in IVD research. We look forward to new studies exploring their potential in the field of IVDD.

5.1.4. Drug inducement for parent cells

Preconditioning parent cells with biochemical factors including autophagy inducers and pro-inflammatory cytokines can modulate the biogenesis and function of EVs [87]. Autophagy is negatively regulated by the mammalian target of rapamycin (mTOR). Rapamycin (Rap), by binding to the Target of Rapamycin Complex 1 (TORC1), inactivates mTOR, thereby enhancing autophagy levels. As mentioned above, exosomal miR-27a alleviated ECM degradation by inhibiting MMP-13. Zhang et al. further demonstrated that NPCs and their exosomes exhibited higher levels of miR-27a through autophagy activation following Rap preconditioning. They proposed that applying the autophagy activator Rap could potentially overcome the challenges associated with obtaining and proliferating nonstem cell-derived exosomes [55]. Similarly, metformin promotes EV biogenesis and secretion by activating autophagy and amphisome-related pathways in MSCs. Researchers discovered that metformin, through activating AMP-activated protein kinase (AMPK), enhances the sorting and transport of inter--α-trypsin inhibitor heavy chain H4 (ITIH4) in vesicular compartments, thus increasing ITIH4 levels in EVs. Once ITIH4 is delivered to NPCs, it participates in anti-aging mechanisms through Akt signaling activation [112]. Zhu et al. discovered that BMSC-derived exosomes inhibited NPC apoptosis, ECM degradation, and fibrosis deposition of NPCs via transferring miR-532-5p. Bioinformatics analysis and dual-luciferase reporter assays identified miR-532-5p targets Ras association domain family member 5 (RASSF5), which is a significant promoter of NPC apoptosis. Following TNF-α treatment (20 ng/ml) of BMSCs for 12 h, the levels of miR-532-5p in exosomes increased, enhancing their inhibitory effect on NPC apoptosis [113].

It is crucial to recognize that the cargo itself can change the physiological processes of parent cells, potentially affecting the composition of other biomolecules within released EVs, thereby negatively influencing their therapeutic efficacy [114].

5.2. Direct EV engineering

Direct engineering of EVs involves loading cargo into isolated EVs using diverse methods like co-cultivation, electroporation, lipofection, sonication, extrusion, click chemistry, and freeze-thaw [115,116]. Compared to parent cell-based engineering methods, direct EV engineering is less technically complex and has been widely employed in developing novel drug delivery systems.

5.2.1. Electroporation

In the process of electroporation, therapeutic agents are mixed with EVs in an electroporation buffer, followed by the application of a high-voltage electrical charge to form transient pores on the EV membrane [117]. This temporary disruption of the EV membrane allows therapeutic agents to permeate into the vesicles. After electroporation, the EVs are incubated at an appropriate temperature to allow membrane recovery. Due to its simplicity, rapid delivery, and minimal dependence on cell type and size [118], bulk electroporation is increasingly being applied for direct EV engineering. For instance, Tang et al. observed that forkhead-box F1 (FOXF1) exhibited the ability to enhance the anti-catabolic and anti-inflammatory properties of NPC. Using bulk electroporation, engineered EVs were employed to deliver plasmids encoding FOXF1 into degenerated NPCs, demonstrating a feasible therapeutic strategy for treating IVDD [119].

5.2.2. Lipofection

Initial lipid transfection techniques utilized a mixture of cationic lipid molecules (e.g., DOTMA or DOTAP) and neutral lipids (e.g., DOPE) for the encapsulation of small nucleic acids [120]. Lipofectamine, a commonly used lipid transfection reagent, forms liposomes in aqueous environments and captures nucleic acids. The positively charged surface of the liposomes facilitates fusion with the negatively charged EV membranes, subsequently releasing the nucleic acids into EVs. For example, Zhang et al. discovered that the deficiency of ataxia-telangiectasia-mutated and Rad3-related protein (ATR) compromised the integrity of the genome and caused the mislocalization of genomic DNA to the cytoplasm, which in turn activated the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) axis, leading to an inflammatory phenotype. To address this, they used lipofection to load ATR-overexpressing plasmids into EVs obtained through ultracentrifugation. These engineered EVs effectively reduced DNA damage-associated NPC senescence and significantly alleviated the progression of IVDD [121].

5.2.3. Sonication

Sonication is a technique where EVs and cargo are mixed in a water-based medium and subjected to ultrasonic waves using a homogenizer ultrasonic probe. This process induces slight deformation of the EV membranes, facilitating cargo loading [122]. Previous studies have demonstrated that miR-378 is capable of restoring autophagy. Hu et al. employed sonication to encapsulate miR-378 mimics in BMSC-derived EVs. The engineered EVs were then loaded into a thread-structural microneedle (T-MN) designed to match the AF structure, allowing for sustained release of the engineered EVs. They confirmed that the T-MN loaded with engineered EVs could adapt to the structure of AF, reducing pathological ECM remodeling, increasing AF cell migration and proliferation, and restoring mitophagy [123].

Despite its potential, direct engineering of EVs faces several limitations. The process of loading may damage the EV integrity and the activity of the cargo. Additionally, extra purification operations are necessary to remove the unencapsulated therapeutic substance, which adds complexity to the process [116,124]. Further studies are required to compare different EV engineering methods in IVDD therapy, ensuring the development of more efficient and reliable treatment.

6. Discussion

EVs are bilipid membrane-capsulated nanovesicles secreted by cells and transfer their contents to regulate the physiological morphology and function of target cells. Serving as carriers of natural signaling molecules, EVs offer advantages such as similarity to parental cell functions, easy editing, convenient storage, and easy separation, leading to their rapid rise in application in regenerative medicine [125]. Compared to other therapies in regenerative medicine, EV therapy is an acellular therapy, overcoming the challenges of potential tumorigenicity of cells and their inability to withstand the harsh IVD environment. The double-layered membrane structure gives EVs a higher bioavailability, stronger biological barrier penetration, and lower immune rejection compared to other drug delivery platforms. Due to their unique properties, many studies in the past decade have revealed the potential of EVs from different sources in treating IVDD. EVs' cargo regulates multiple downstream targets with distinct biological functions and produces diverse therapeutic outcomes in the disc cells.

The traditional view holds that there are several unresolved issues regarding the application of EVs in IVD diseases: (1) Lack of standardization in EV preparation, (2) Inability to accomplish EV large-scale production, (3) Challenges in EV delivery routes and concentrations in the IVD, and (4) Identification of active molecules in EVs. With increasing attention to EV research and the development of EV technologies, these issues are gradually being addressed.

Firstly, there are shortcomings in the preparation of EVs. So far, six types of isolation strategies have been reported for experimental EVs, including microfluidic technologies, size-exclusion chromatography, polymer precipitation based on charge neutralization, ultrafiltration, immunoaffinity capture, and ultracentrifugation [126]. However, the lack of standardized isolation processes in the past has led to uncertainty regarding the biological effects and safety of EVs. Therefore, minimal information for studies of extracellular vesicles (MISEV) 2023 updated by the International Society for EVs (ISEV) explicitly provides recommendations for sample collection, pre-processing, isolation, collection, characterization, and storage during EV preparation from different sources and using different isolation methods [38]. This is expected to enhance the accuracy and reliability of future EV research. Additionally, the heterogeneity of cargo within different batches of EVs introduces further complications to EV-based therapies. Variability in the active and inactive components of EV batches can lead to inconsistent therapeutic outcomes and, in some cases, unexpected side effects [127]. To address these challenges, the use of iMSCs has been proposed as a potential solution. iMSCs can be produced on a large scale under standardized in vitro conditions, significantly reducing donor variability and providing a more uniform EV product [128].

Secondly, high production costs and low yields are two major bottlenecks in EV therapy. Static systems based on flasks in cell culture rooms are often used for laboratory-scale parent cell cultivation. Disappointingly, the amount of exosomal protein produced in 1 mL of culture medium is less than 1 μg, far less than the effective dose of exosomes in vivo experiments (10–500 μg exosomal protein/mouse) and the required amount of exosomes for patients in clinical trials (0.5–1.4 $\times$ 10^{11} per person) [129]. Nowadays, large-scale cultivation methods, including hollow-fiber membranes, microcarriers, and microfiber-bed types, are dedicated to optimizing separation and enrichment methods and developing standardized processes and regulations, to enable large-scale EV production [130]. Hollow-fiber bioreactors used for cell culture have recently been reported for the large-scale production of EVs. These bioreactors support enormous quantities of high-density cells without the need for cell splitting and passaging while retaining large secretory products such as exosomes [131,132]. It is believed that shortly, large-scale and efficient production of EVs will become possible, enabling their use in clinical trials in the drug development process.

In addition, the administration route and optimal dosage for EV injection are not yet clear and require further research. Currently, there are mainly two approaches for EV therapy for IVDD: systemic administration and local treatment of the IVD [41].

For systemic administration, as the lack of blood flow in IVD and EVs mainly accumulate in the liver, lungs, and kidneys, it is necessary to consider how to upgrade the EV concentration in the IVD and whether it causes adverse reactions in other organs [133]. Nowadays, with the maturity of gene editing technology and click chemistry, engineered EVs can express targeting ligands such as cavin-2 on the EVs membrane to promote the absorption of EVs by receptor cells, thereby improving efficacy [91]. It is believed that in the future, the issue of EV absorption in the IVD will be effectively resolved.

For local EV therapy, repeated IVD injections may cause damage to the annulus fibrosus. To overcome this problem, researchers have combined tissue engineering with EVs. Hydrogels, as three-dimensional hydrophilic polymers, are regarded as ideal implants for the treatment of IVDD due to their excellent biocompatibility and ability to mimic the water-absorbing and retaining properties of natural nucleus pulposus tissue. Compared to other biomaterials, hydrogels not only create a protective environment that supports the survival and function of EVs but also offer highly tunable mechanical properties and biodegradability. Utilizing hydrogels as a biomaterial scaffold for EVs enables the sustained release of EVs, thereby enhancing therapeutic efficacy while leveraging these inherent advantages [134]. In one study, researchers collected adipose-derived MSC exosomes and loaded them on an ECM hydrogel, achieving the effect of supplementing ECM, providing an NP microenvironment, and ensuring continuous release of exosomes [135]. However, the application of hydrogels in IVDD therapy still faces several challenges. For instance, no single hydrogel is perfect: natural hydrogels often lack ideal mechanical properties and pore structures, while synthetic hydrogels can have poor biocompatibility. Additionally, a key challenge is designing hydrogel scaffolds that can accommodate the differing biological structures and mechanical strengths of the NP and AF tissues [136].

In the future, EV therapy is expected to be more combined with tissue engineering, genetic engineering, metabolic engineering, and click chemistry techniques, becoming an extension of cell therapy. The intersection of disciplines is bound to overcome the inherent risks of cell therapy and demonstrate stronger targeting, tissue compatibility, and lasting regenerative repair capabilities in the IVD.

Finally, a growing number of studies have reported the different functions and mechanisms of action of EVs carrying different cargos in IVDD therapy, which have been thoroughly expatiated in this review. However, the differences in cargo expression types and concentrations between EVs from different sources are still unknown. In the field of cancer, researchers have developed a highly integrated electrochemical platform for the molecular analysis of exosomes in blood samples. The platform's metal–organic framework-functionalized sensing interface can collect exosomes from biological fluids without the need for additional purification steps, and analyze exosome protein and RNA markers by its sensing strategy. It successfully identified breast cancer patients with 100 % sensitivity and specificity [137]. In the realm of IVDD, there is a paucity of studies investigating the potential of diagnosing IVDD and predicting its prognosis by analyzing variations in the types and concentrations of EVs in various body fluids. This promising area of research warrants significant attention in future studies.

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Declaration of competing interest

The authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

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