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REVIEW

Exosomes and Renal Fibrosis: Diagnostic Value, Therapeutic Potential and Challenges

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Abstract: Renal fibrosis is a key pathological process in the progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD), characterised by irreversible damage to the renal parenchyma. Currently, effective curative treatments are lacking. Exosomes, double-layer phospholipid vesicles containing bioactive components such as proteins, lipids, and nucleic acids, play a pivotal role in intercellular communication. Under physiological conditions, exosomes contribute to kidney development (eg regulating of ureteric bud branching and nephron formation) and maintenance of cellular homeostasis (eg protection of the glomerular filtration barrier and regulation of electrolyte balance). In pathological conditions, damaged renal tubular epithelial cells (RTECs) and other renal cell types release exosomes carrying pro-fibrotic factors (eg miR-21, TGF-β), which activate fibroblasts and facilitate excessive extracellular matrix (ECM) deposition, thereby accelerating the fibrotic process. Exosomes possess significant diagnostic value, as their protein components (eg Cp and CD2AP in urinary exosomes) and RNA cargo (eg lncRNA, miRNA, circRNA) may serve as biomarkers for renal function impairment. Therapeutically, exosomes derived from bone marrow, adipose tissue, umbilical cord, and urine can delay fibrosis through multiple mechanisms, including anti-inflammatory effects, antioxidant activity, promotion of angiogenesis, and regulation of signalling pathways (eg NOTCH, AKT). Engineered exosomes exhibit enhanced targeting and delivery efficiency through endogenous or exogenous loading methods, thereby further improving therapeutic efficacy. However, current research faces challenges including inconsistent methods of exosome isolation and purification, absence of standardised identification protocols, insufficient stability, and barriers to clinical translation. This review summarises the current progress in exosome research related to renal fibrosis, covering physiological and pathological roles, diagnostic and therapeutic potential, and existing challenges, aiming to facilitate translation from basic research to clinical practice and to provide novel strategies for precise diagnosis and treatment of renal fibrosis.

Keywords: exosomes, renal fibrosis, CKD, ECM, MSCs

Introduction

Renal fibrosis is a critical marker and pathway in the progression of chronic kidney diseases (CKD). It is a major determinant of renal failure and a reliable prognostic indicator. CKD is a global public health issue, which is defined by an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m², persisting for more than three months.[1] As the global population ages, the burden of CKD and its associated risk factors continues to increase. Predictions indicate that by 2050, the prevalence of CKD stages G3–G5 in certain regions may exceed 10%.[2] Various factors, such as diabetes, hypertension, glomerulonephritis, obstructive nephropathy and others can cause irreversible damage to kidney structure and function.[3,4] In addition, patients with polycystic kidney disease (PKD) often experience worsening kidney function due to delayed diagnosis, accompanied by a unique spectrum of PKD1 gene mutations, suggesting that genetic background and regional differences may contribute to CKD progression.[5] Numerous studies have confirmed that after kidney injury, the inflammatory response is rapidly activated, leading to infiltration of macrophages, key effector cells, in the renal interstitium, releasing cytokines such as TGF-β and PDGF. These cytokines stimulate the activation,

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proliferation, and migration of fibroblasts, significantly promoting the excessive synthesis and abnormal deposition of extracellular matrix (ECM).[6] Due to the asymptomatic nature of early-stage CKD and the diagnostic reliance on categorical variables (eg eGFR), developing predictive models remains challenging.[7] Patients in progressive disease stages typically present with worsening uremia, electrolyte abnormalities, anaemia, mineral and bone metabolism disorders, and acid-base imbalance.[8] Compared to organs like the liver, the kidney has a limited self-repair capacity. Currently, renal replacement therapy (RRT) is the preferred treatment option, but it is costly and associated with numerous complications.[9] Therefore, identifying more effective treatments to delay renal fibrosis remains a major challenge.

The concept of exosomes was first discovered by Pan and Johnstone in 1983 in sheep reticulocytes. The term "exosome" was introduced by Johnstone in 1987.[10,11] Exosomes are double-layer phospholipid vesicles secreted by cells. They are widely present in various body fluids and cell culture environments, with unique structural and functional characteristics. They contain diverse bioactive components, including proteins, lipids, and nucleic acids, enabling signal transmission and intercellular communication.[12] In recent years, the role of exosomes in CKD has become an important research focus. Increasing evidence indicates that exosomes serve as key mediators of intercellular communication both locally in the kidneys and systemically, significantly influencing the pathogenesis, progression, and outcomes of CKD. On one hand, exosomes released by intrinsic renal cells (eg podocytes, epithelial cells, mesangial cells) and infiltrating immune cells regulate the biological behaviors of neighbouring or distant cells by transmitting specific signalling molecules. On the other hand, circulating exosomes participate in pathological processes such as inflammatory infiltration, vascular lesions, and metabolic disorders.[13] Furthermore, exosomes from diverse sources have distinct and crucial roles in kidney development, maintenance of physiological function, auxiliary diagnosis, prognostic assessment, and potential targeted therapies, due to the specific molecular cargo they carry.[14]

Drawing on a comprehensive literature search of basic and clinical research, this article summarises the latest advances regarding exosome biogenesis, their physiological and pathological roles in renal fibrosis, exosome-based therapeutic strategies, and current challenges in research and clinical translation. The aim is to provide deeper insights into the molecular mechanisms underlying CKD and to promote the development of novel diagnostic and therapeutic methods.

Sources, Occurrence, and Differentiation Process of Exosomes

Exosomes, one of the three types of extracellular vesicles (EVs), have a diameter of approximately 30–150 nm and possess a lipid bilayer with biological activity. They consist of various proteins (such as heat shock proteins [HSPs] and tetraspanins), lipids (such as ceramide and cholesterol), and nucleic acids (DNA, mRNA, and miRNA).[15] Exosomes can be classified as animal-derived or plant-derived. Many types of normal cells can produce exosomes, including mesenchymal stem cells (MSCs), human umbilical vein endothelial cells, T cells, macrophages, and dendritic cells (DCs) (Figure 1).[16]

The synthesis of exosomes involves the invagination of the cell membrane and the formation of intracellular multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs).[17] The invagination of the plasma membrane forms a cup-shaped structure, creating early sorting endosomes(ESEs).[18] Some ESEs may merge with pre-existing ESEs, and structures such as the endoplasmic reticulum also participate in ESE formation. ESEs then mature into late sorting endosomes (LSEs). After further invagination, the contents are encapsulated to form multiple ILVs, and the LSEs evolve into MVBs. Finally, mature MVBs either fuse with lysosomes for degradation or with the plasma membrane to release ILVs, which are known as exosomes.[19] The formation of ILVs is the core process in exosome synthesis. Recent studies have proposed various mechanisms for ILVs formation, which can be broadly categorized into the classical endosomal sorting complex required for transport (ESCRT)-dependent pathway and ESCRT-independent pathways.[20] In the classic ESCRT- dependent pathway, ESCRT consists of ESCRT-0, -I, -II, -III complexes, the ATPase VPS4, and various auxiliary components that work together synergistically. First, ESCRT-0, composed of hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) and signal transducing adaptor molecule (STAM), recognizes and binds to ubiquitinated cargo proteins. The Hrs-STAM complex then binds to phosphatidylinositol 3-phosphate (PI3P) on the early endosomal membrane via the FYVE domain on Hrs, directing it to the membrane. Ubiquitin subsequently recruits

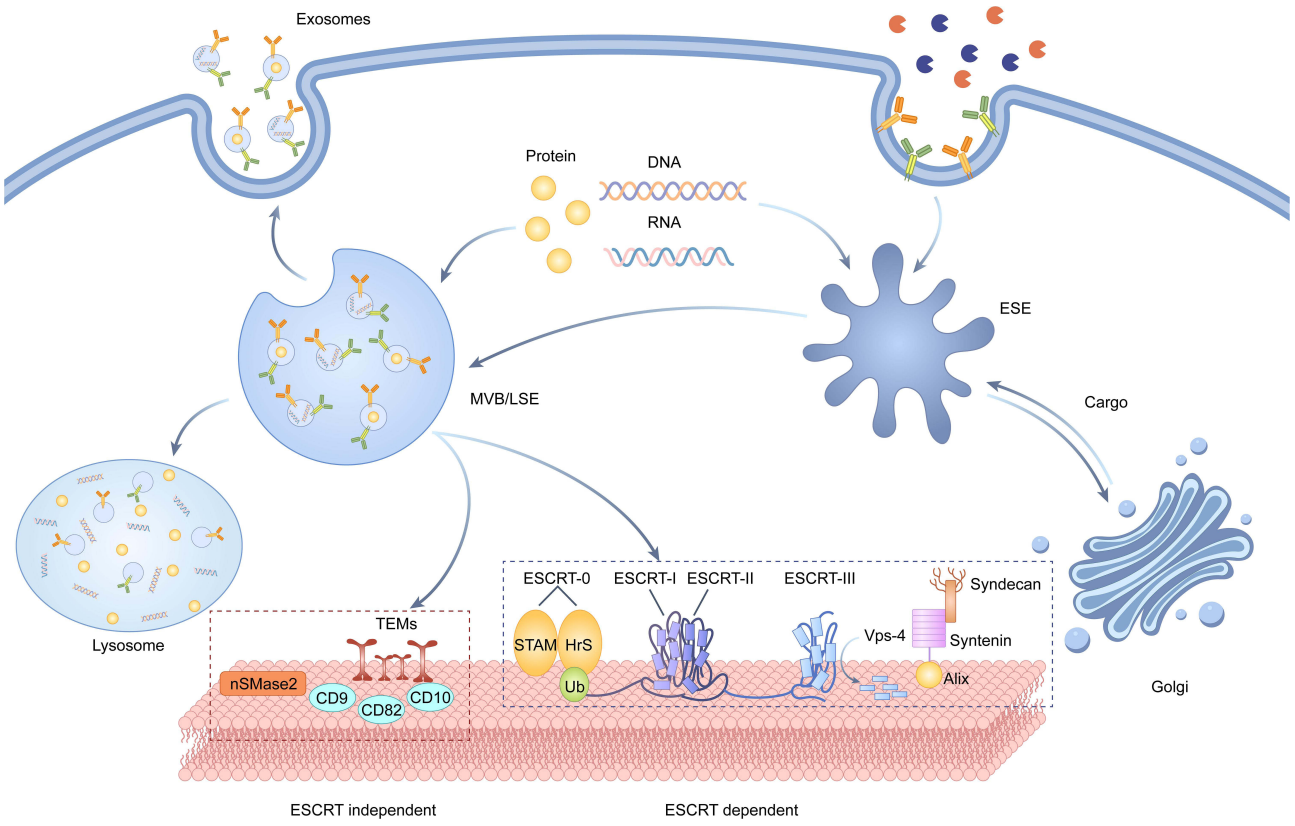

Figure 1 Biogenesis and secretion mechanism of exosomes.
Notes: The membrane invaginates to form ESEs containing entotic substances. The Golgi apparatus and other structures also contribute to the formation of ESEs. Substances are then sorted into the endosomal cavity to form LSEs, which mature into MVBs. There are two pathways for the formation of polyvesicles: ESCRT-dependent pathway: Complexes sequentially function to recognize and recruit Ub proteins and other cargo molecules. Syndecan, Syntenin, Alix, and others participate, ultimately forming MVBs/LSEs containing exosomes. ESCRT-independent pathway: Involving nSMase2 and TEMs, this pathway promotes the formation of MVBs/LSEs through different mechanisms. MVBs/LSEs containing exosomes fuse with lysosomes, leading to degradation of their contents. Unfused MVBs/LSEs fuse with cell membranes, releasing exosomes into the extracellular environment.
Abbreviations: ESCRT, endosomal sorting complex required for transport; ESE, early sorting endosomes; LSEs, late secreting endosomes; MVB, multivesicular bodies; nSMase2, neutral sphingomyelinase 2; TEMs, tetraspanin-enriched microdomains.

ESCRT-I to the membrane by binding to the structural domain of ESCRT-I, which in turn recruits ESCRT-II through the Vps28 and EAP45 subunits. Finally, ESCRT-III is recruited by ESCRT-II and mediates the scission of ILVs, after which the auxiliary protein VPS4 dissociates the ESCRT-III complex for recycling.[21] In addition, the Syndecan-Syntenin-Alix pathway contributes to ILVs synthesis. In the absence of ubiquitinated cargo and the ESCRT-0 complex, the Alix pathway can recruit the remaining ESCRT proteins to participate in ILVs synthesis.[22] Syndecan is a transmembrane heparan sulfate proteoglycan whose carboxyl terminal binds to the PDZ domain of Syntenin, recruiting Syntenin to the cytoplasmic membrane. The N-terminal of Syntenin then interacts with Alix, facilitating the interaction between ESCRT-I and ESCRT-III proteins and promoting Syndecan's role in ILVs generation and cargo sorting.[23] Majer et al reported that the membrane protein UNC93B1 recruits Syntenin to promote the sorting of UNC93B1-TLR7 complexes into ILVs.[24] Research by Zhang et al found that the small GTPase ADP-ribosylation factor 6 (ARF6) and its effectors, phospholipase D2 (PLD2), Ral GTPase, and Src homology 2-containing protein tyrosine phosphatase 2 (Shp2), regulate ILVs formation mediated by the Syndecan-Syntenin-Alix pathway by modulating Syntenin phosphorylation.[25] The ESCRT-independent pathway refers to the formation and release of ILVs that do not require the involvement of the four ESCRT protein complexes. Currently, the most studied pathways are the ceramide and tetraspanin pathways. Neutral sphingomyelinase 2 (nSMase2) is a key enzyme that converts sphingomyelin into ceramide.[26] It is proposed that ceramide induces the aggregation of microdomain structures, promoting their budding. Additionally, some studies suggest that the conical structures formed by ceramide can induce spontaneous negative membrane curvature.[27,28] However, the specific mechanism by which the ceramide pathway mediates ILVs formation remains unclear.[29,30] Tetraspanins, such as

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CD63, CD9, CD10, and CD82, form a protein family that interacts with cytoplasmic proteins to create tetraspanin-enriched microdomains (TEMs), which facilitate ILVs formation.[31]

Exosomes and Renal Fibrosis

Physiological Role of Exosomes in the Kidneys

Kidney Development

The kidneys, as vital organs in the urinary system, undergo a complex morphogenetic process during development. The pronephros stage occurs early, degenerates rapidly, and is not functional. During the mesonephros stage, some structures persist through degeneration and exhibit limited excretory functions. The extension and growth of the ureteric bud (UB) mark the transition to the metanephros stage, which forms the permanent kidneys. By 18–20 weeks of gestation, the basic kidney structure is established, and development continues until 32–36 weeks.[32] Exosomes are involved in various stages of kidney development, including ureteric bud branching, metanephric mesenchyme (MM) accumulation and differentiation, nephron unit formation, and overall structural shaping. In the early stages of kidney development, miRNAs such as miR-17-5P and miR-26b-5P, derived from UB cells and embryonic kidney UB stem cells, regulate secondary inductive effects in organogenesis. They modulate the Wnt pathway and enrich extracellular matrix proteins, influencing organ formation. Additionally, exosomes from the UB significantly enhance the viability of MM cells and their ability to form kidneys. These exosomes promote the orderly differentiation of cells and support the gradual formation of structures, such as renal tubules, in a three-dimensional arrangement. This process enables the kidneys to acquire essential physiological functions, such as filtration and reabsorption. Furthermore, exosomes regulate the arrangement of ureter wall cells and the remodeling of the extracellular matrix, ensuring that the ureters maintain appropriate diameters and morphological characteristics for effective peristalsis, which facilitates urine transport (Figure 2).[33]

Communication Function

In the kidneys, exosomes function as EVs during cell exocytosis. They help eliminate intracellular waste and maintain cellular homeostasis by carrying substances such as nucleic acids, proteins, and lipids. Once released, exosomes communicate between kidney cells through various mechanisms, including adhesion to the surface of target cells, fusion with the target cell membrane, or internalization by target cells.[34] Podocytes and endothelial cells, which form the glomerular filtration barrier, are closely adjacent. Exosomes secreted by these cells help maintain the structural integrity of the filtration barrier. Podocyte-derived exosomes carry various signaling factors, including Wilms tumor 1 (WT1), Podocin, Actinin, alpha 4 (ACTN4), CD2 associated protein (CD2AP), and Nephrin. These factors are involved in intercellular communication, maintaining the homeostasis and morphology of podocytes and endothelial cells, and protecting glomerular filtration function. Endothelial cell-derived exosomes carry miR-192-5p, which regulates the expression of nephronectin (NPNT) in podocytes, maintaining the normal structure and permeability of the glomerular basement membrane (GBM).[35] Additionally, miR-4455 secreted by mesangial cells can be transferred to podocytes, indirectly affecting podocyte autophagy by regulating unc-51-like kinase 2 (ULK2).[36] In the renal tubules, cells in different segments achieve gradient reabsorption of substances such as glucose, amino acids, and electrolytes through molecules transmitted by exosomes. Proximal tubular epithelial cells transport sodium-hydrogen exchanger 3 (NHE3) to distal tubular epithelial cells via exosomes, regulating reabsorption in the distal tubule and maintaining electrolyte balance.[37] Under conditions of dehydration or a high-salt diet, exosomes secreted by collecting duct cells enhance water reabsorption. They maintain fluid balance and osmotic pressure stability by regulating the expression and distribution of aquaporin 2 (AQP2).[38] Some immune cells in the kidneys also secrete exosomes to maintain renal immune homeostasis. DCs, classified into mature dendritic cells (mDCs) and immature dendritic cells (imDCs), are antigen-presenting cells. Research has shown that exosomes from mDCs carrying Programmed Death-Ligand 1 (PD-L1) can bind to the PD-1 receptor (PD-1r) on T cells, inhibiting T cells activation and preventing autoimmune inflammation.[39] Meanwhile, imDCs induce antigen-specific T cell tolerance. Exosomes from imDCs promote T cell differentiation and enhance renal immune tolerance by delivering miR-682, which negatively regulates ROCK2. Additionally, Li et al found that macrophage-derived exosomes can regulate endothelial cell m6A methylation and cellular inflammatory responses by transferring methyltransferase-like 14 (METTL14) (Figure 2).[40]

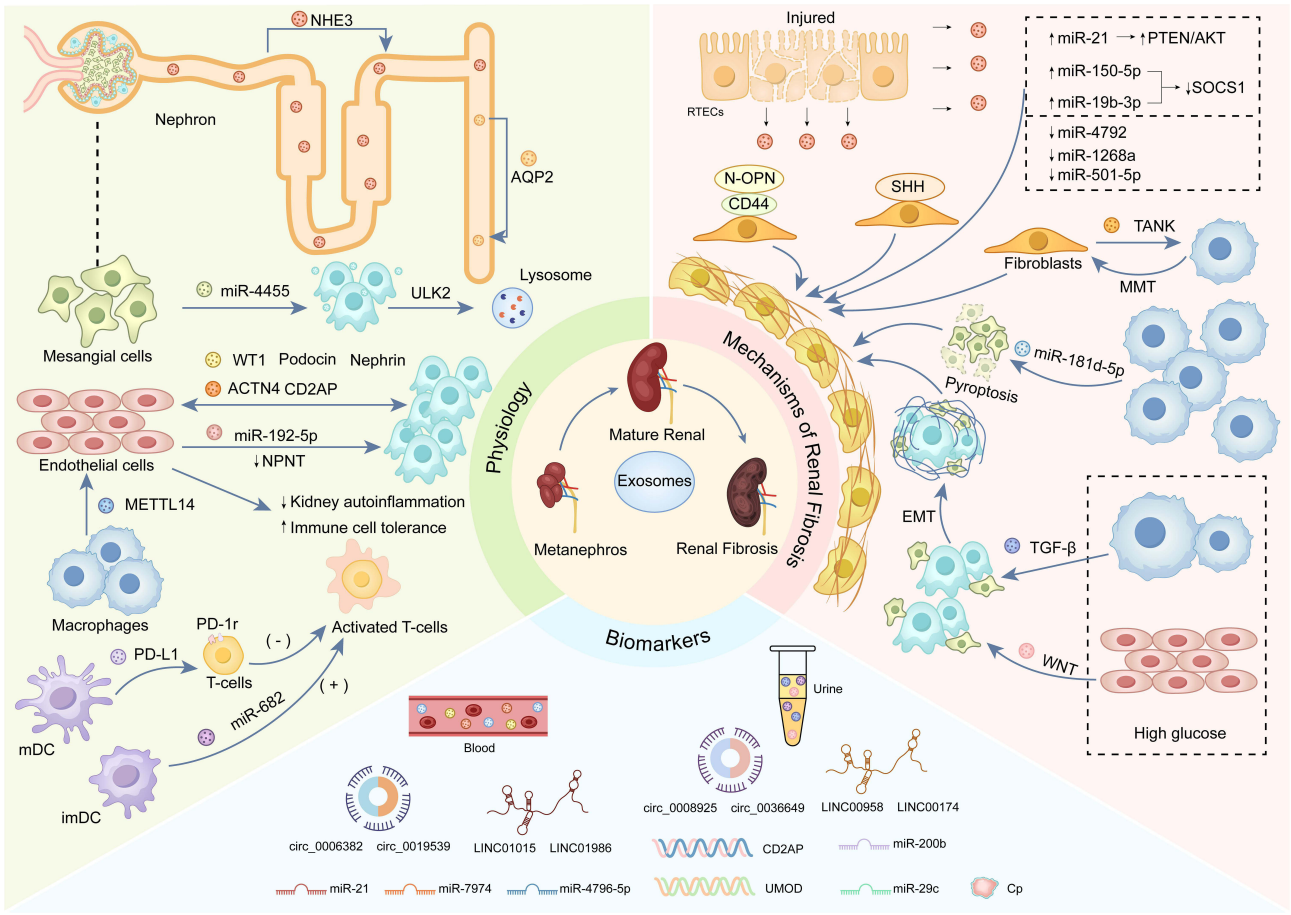

Figure 2 The processes of kidney development and fibrosis involve exosomes.
Notes: Physiological role of exosomes: Intrinsic renal cells communicate with each other through the transmission of substances such as miR-4455, WT1, and miR-192-5p. Exosomes from collecting duct cells promote water reabsorption. Exosomes derived from dendritic cells and macrophages can enhance cellular immunity and alleviate renal inflammation. Mechanisms of renal fibrosis involving exosomes: After injury to the renal tubular epithelial cells (RTECs), exosomes released carry N-OPN/SHH, which binds to fibroblasts and promotes the release of exosomes from fibroblasts, thereby mediating the epithelial-mesenchymal transition (EMT) of macrophages. The miR-181d-5p in macrophage exosomes accelerates the pyroptosis of mesangial cells. In a high glucose (HG) environment, exosomes promote the deposition of extracellular matrix (ECM), leading to the progression of fibrosis. Biomarkers: Exosomes in blood and urine contain specific proteins and various types of RNA.

Exosomes Involved in the Formation of Renal Fibrosis

Under stress conditions such as inflammatory stimulation, oxidative stress, and hypoxia, the secretion of exosomes released by damaged renal tubular epithelial cells (RTECs) significantly increases. This mechanism is related to the upregulation of components in the ESCRT protein complex or proteins involved in vesicle transport, such as tumor susceptibility gene 101 (TSG101) in ESCRT-I, Rab, and others.[41] Chen et al suggest that β-catenin in RTECs promotes exosome secretion and transports the N-terminal fragment of osteopontin (N-OPN) to fibroblasts. There, it binds to CD44, regulating cell adhesion, proliferation, and apoptosis, thereby further promoting fibrosis.[42] After kidney injury, Sonic hedgehog (SHH) is specifically induced in RTECs and targets fibroblasts via exosome-mediated pathways, promoting their activation.[43] miRNA array analysis shows that the expression of miR-21, miR-150-5p, and miR-19b-3p is significantly increased in RTEC-derived exosomes. miR-21 targets the PTEN/AKT signaling pathway, while miR-150-5p and miR-19b-3p induce macrophage polarization towards the M1 type by inhibiting suppressor of cytokine signaling 1 (SOCS1), accelerating renal fibrosis.[44–46] However, some miRNAs, such as miR-4792, miR-1268a, and miR-501-5p, are downregulated in exosomes after kidney injury, and their mechanisms remain unclear.[47] Wu et al found that under high glucose conditions, exosomes from glomerular endothelial cells play a role in crosstalk, promoting the epithelial-mesenchymal transition (EMT) of glomerular mesangial cells and podocytes via the Wnt pathway.[48] High glucose also increases the secretion of exosomes from macrophages, activating inflammatory responses in renal tissue

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through the TGF-β/Smad3 pathway. This leads to excessive mesangial cell proliferation, matrix deposition, and inflammatory infiltration.[49] Additionally, exosomes from macrophages carry miR-181d-5p in renal autoimmune diseases, targeting BCL-2 in mesangial cells and exacerbating cell pyroptosis.[50] Exosomes from myofibroblasts promote macrophage-to-myofibroblast transition (MMT) via the TANK-binding kinase 1 (TBK1) signaling pathway, further facilitating renal fibrosis (Figure 2).[51]

Exosomes as Biomarkers for Diagnosing Renal Fibrosis

With ongoing research into exosomes, their potential as biomarkers is becoming increasingly clear. Exosomes are present in various body fluids, such as blood, urine, and saliva, and are characterized by ease of access and minimal invasiveness.[52] The lipid bilayer structure of exosomes provides stability in fluids like urine and blood, protecting their contents from degradation by enzymes and reducing errors caused by sample collection or biomolecular degradation.[53] Due to their diverse origins, exosomes contain substances with unique characteristics, offering precise evidence for early disease diagnosis and detection. In kidney disease research, exosomes are commonly extracted from blood and urine as biomarkers for disease prediction.

Urinary exosomes contain kidney-specific protein molecules and markers, making them easy to isolate and a growing focus for non-invasive examinations. Ceruloplasmin (Cp), a copper-containing α2-glycoprotein with ferroxidase activity, is primarily involved in iron metabolism and protecting cells from oxidative stress. In the urine exosomes of patients with CKD, Cp expression is 10–20 times higher than in the control group. Animal model studies have shown that increased Cp expression in urinary exosomes occurs before proteinuria onset, suggesting it could serve as an early marker for kidney disease.[54] CD2AP, a specific protein in renal podocytes, participates in multiple signaling pathways associated with kidney diseases. Research has demonstrated a significant negative correlation between CD2AP content in urinary exosomes and 24-hour urinary protein, BUN, tubulointerstitial fibrosis, and glomerulosclerosis coefficient, suggesting its utility as a biomarker for predicting renal function.[55]

The RNA species carried by exosomes are diverse, and their potential as biomarkers is gradually being explored. Clinical studies involving patients with diabetic nephropathy (DN) have demonstrated that LINC00958 and LINC00174 are significantly upregulated in urinary exosomes compared with those from healthy controls. The expression of LINC00958 negatively correlates with eGFR, whereas LINC00174 expression positively correlates with urinary protein levels.[56] Furthermore, decreased expression of LINC01619 in renal tissue significantly correlates with proteinuria and deterioration of renal function, possibly acting by sponging miR-27a and thereby regulating FOXO1-mediated oxidative stress and podocyte injury. Therefore, whether differential expression of LINC01619 in urine exosomes has clinical implications requires further validation.[57] Circular RNA (circRNA) is a non-coding RNA species with a covalently closed loop structure, characterised by a long half-life and strong nuclease resistance. Research by Cao et al found increased expression of circ_0008925 in urinary exosomes of patients with renal fibrosis, positively correlating with serum creatinine, blood urea nitrogen, renal tubulointerstitial fibrosis scores, and glomerulosclerosis scores.[58] Conversely, circ_0036649 expression in urinary exosomes was reduced, negatively correlating with the aforementioned indicators.[59] In urinary exosomes from CKD patients, expression levels of the miR-29 and miR-200 families are significantly lower compared to healthy controls. Furthermore, there is a significant negative correlation between the severity of tubulointerstitial fibrosis and expression levels of miR-29c and miR-200b. Notably, miR-200b expression is significantly reduced in moderate and severe cases compared to mild cases, indicating that miR-29c and miR-200b may serve as indicators of fibrosis severity and renal function impairment.[60,61] The mRNA of UMOD, secreted by RTECs and encoding uromodulin, is notably elevated in the DN patient group. Previous studies have confirmed that UMOD overexpression typically coincides with abnormal expression of the Na+/K+/2Cl- cotransporter NKCC2. In this study, the mRNA SLC12A1, which encodes NKCC2, is also significantly elevated in DN patients, paralleling UMOD expression and associating with proteinuria. This elevation suggests that increased UMOD and SLC12A1 expression in urine-derived exosomes may indicate worsening kidney injury.[62]

In recent years, the prevalence of blood exosomes, particularly circulating exosomes, has increased. These can be categorized into serum and plasma exosomes based on their blood components.[63] Ana et al collected plasma samples from 96 patients with systemic lupus erythematosus (SLE), including 23 cases of lupus nephritis (LN), as well as

25 healthy controls, to extract exosomes. Using Small RNA-Seq library preparation techniques for high-throughput sequencing, they found that LINC01015, LINC01986, AC087257.1, and AC022596.1 were significantly upregulated. Bioinformatics predictions suggest these may regulate actin cytoskeleton dynamics and podocyte adhesion, migration, and spreading by targeting the GTSYS family proteins and insulin-like growth factor receptors, among other pathways.[64] In serum exosomes of diabetic patients, circ_0006382 and circ_0019539 expression was significantly downregulated. Bioinformatics analysis suggests a possible link to the FGF9 gene and EMT of RTECs.[65] Studies by Saejong indicate that approximately 65% of patients develop chronic allograft dysfunction within 24 months after kidney transplantation, primarily characterized by renal interstitial fibrosis and tubular atrophy (IF/TA). In patients with IF/TA grades II–III, the levels of miR-21 in plasma exosomes are significantly higher than in those with grade I, whereas no significant difference is observed in whole plasma miR-21 levels. Thus, miR-21 in plasma exosomes could differentiate between IF/TA grade I and grades II–III, addressing the limitations of traditional kidney injury biomarkers (eg creatinine, blood urea nitrogen, proteinuria) in diagnosing IF/TA grades.[66] Furthermore, the expressions of miR-4796-5p and miR-7974 are significantly higher in various kidney diseases (such as IgAN, DN, LN), with KEGG analysis suggesting their association with pathways like mTOR and PI3K-AKT, potentially serving as biomarkers for multiple kidney diseases.[67]

Exosomes derived from peritoneal dialysis effluent (PDE) predominantly originate from mesothelial cells and demonstrate significant potential as biomarkers for kidney disease. A study involving children with focal segmental glomerulosclerosis (FSGS) undergoing peritoneal dialysis (PD) identified, for the first time, a core proteomic profile consisting of 40 proteins capable of accurately distinguishing FSGS from other primary kidney diseases through proteomics combined with machine learning analysis. Among these proteins, annexin A13 (ANXA13) was identified as the most promising upregulated biomarker, and its significant elevation in FSGS patients was validated by ELISA in an independent cohort of serum samples. Additionally, the study found a significant negative correlation between the expression level of protein tyrosine phosphatase 4A1 (PTP4A1) and the duration of PD, suggesting its utility as a sensitive indicator of damage caused by prolonged exposure to the dialysis environment (Figure 2).[68]

Exosomes and Oligonucleotide-Based Gene Therapy

Currently, gene therapy strategies for renal fibrosis are becoming increasingly diverse. Among these, oligonucleotide-based gene therapy, which exerts inhibitory effects by targeting specific DNA or RNA sequences through artificially synthesised short-chain DNA, RNA, or their analogues, represents a novel form of conventional gene therapy.[69] Common oligonucleotide therapeutic approaches include small interfering RNA (siRNA), antisense oligonucleotides (ASOs), microRNA (miRNA), and aptamers. Meanwhile, the application of exosomes as molecular delivery vehicles for precise therapeutic targeting has gained rapid attention in recent years, emerging as a highly promising therapeutic strategy (Table 1).[70]

siRNA is a double-stranded RNA molecule, capable of specifically targeting and degrading mRNA. Its antisense strand binds to the Argonaute2 protein (AGO2), forming the RNA-induced silencing complex (RISC).[71] siRNA can also be engineered in various forms, such as long non-coding RNA (lncRNA) for transcriptional repression or short hairpin RNA (shRNA) typically delivered into cells via viral vectors.[72] A clinical Phase II study investigated Teprasiran, which exerts a preventive effect against acute kidney injury (AKI) in high-risk patients undergoing cardiac surgery by transiently inhibiting P53. This study demonstrated that postoperative intravenous administration of Teprasiran effectively reduced the incidence of AKI; however, it did not address the impact of Teprasiran on fibrosis markers.[82] Furthermore, siRNA-based therapeutic approaches for renal fibrosis remain in preclinical stages. For instance, siRNA-mediated knockdown of the N6-methyladenosine (m6A) methyltransferase METTL3 in cellular and mouse models alleviated fibrosis induced by TGF-β and unilateral ureteral obstruction (UUO) through inhibition of the m6A methylation process.[83] Acyl-CoA synthetase long-chain family member 4 (ACSL4) primarily participates in the biosynthesis of polyunsaturated fatty acid (PUFA)-phosphatidylethanolamines (PE). Targeted inhibition of ACSL4 using siRNA promoted peroxide depletion in renal tissue, thus inhibiting ferroptosis-induced fibrosis.[84] However, due to siRNA's short half-life and duration of action, approximately five minutes in the bloodstream, and because most siRNA molecules are degraded by lysosomes upon cellular entry, with only 1–2% exerting their effects in the cytoplasm, further research is necessary to fully explore siRNA's therapeutic potential.

Table 1 Comparisons of Exosomes and Oligonucleotides in Therapy

Name	Structure	Target Specificity	Mechanism	Expandability	Challenge	Reference
siRNA	Double-stranded RNA 21–23 bp	mRNA	RISC-mediated sequence-specific mRNA cleavage	Enhanced cellular uptake achievable through LNP delivery systems	1. Lysosomal degradation 2. Off-target effects 3. Limited extra-hepatic delivery efficiency	[71,72]
ASO	Single-stranded DNA 15–20 nt	mRNA	RNase H-mediated mRNA degradation or steric blockade of splicing machinery or translation	Compatibility with CRISPR systems for exon-skipping applications	1. Dose-limiting nephrotoxicity 2. Poor extrahepatic tissue 3. biodistribution	[73,74]
miRNA	Single-stranded RNA 20–22 nt	mRNA	Translational repression or mRNA destabilization through 3′-UTR binding	Broad therapeutic applicability across multiple disease pathways	1. Interference with endogenous miRNA networks 2. Unclear long-term regulatory consequences	[75–77]
Aptamer	Single-stranded DNA/ RNA 25–80 nt	Specific proteins	High-affinity target protein binding for functional antagonism or agonist activity	Bivalent/bispecific constructs enhance targeting; Improved SELEX efficiency	1. Rapid renal clearance 2. Nuclease susceptibility 3. Off-target binding to serum proteins	[78,79]
Exosomes	Endogenous nanovesicles 30–150 nm	Cells	Membrane fusion-mediated cytosolic delivery of cargo	Engineered loading enhances drug loading efficiency and targeting specificity	Heterogeneity in isolation methods	[80,81]

Abbreviations: ASO, antisense oligonucleotide; bp, base pair; CRISPR, clustered regularly interspaced short palindromic repeats; LNP, lipid nanoparticle; mRNA, messenger RNA; nt, nucleotide; nm, nanomete; UTR, untranslated region; RISC, RNA-induced silencing complex; siRNA, small interfering RNA; SELEX, systematic evolution of ligands by exponential enrichment.

ASOs are synthetic, short, single-stranded nucleic acids, which inhibit gene expression by targeting mRNA sequences.[73] ASOs generally exert their effects through two mechanisms: firstly, complementary binding to mRNA forms DNA-RNA heteroduplexes, leading to recognition and degradation of the RNA strand by RNase H; secondly, binding to pre-mRNA influences alternative splicing.[74] Preclinical studies have shown that an ASO-based approach can interfere with the fibronectin extra domain A (EDA+) splice variant exon in renal proximal tubular cells, thus inhibiting its activation of TGF-β and alleviating renal fibrosis. The homodimeric transmembrane glycoprotein endoglin (ENG) typically serves as a co-receptor for the TβRI-TβRII complex, with the long ENG isoform promoting fibrosis and the short ENG isoform inhibiting fibrosis. ASO-targeted therapy aimed at ENG can increase the short ENG/ total ENG ratio and reduce ECM deposition by inhibiting TGF-β signaling.[85,86] Currently, certain ASO-based drugs have advanced to clinical application for diseases including Duchenne muscular dystrophy and vascular disorders. However, unresolved issues such as hepatotoxicity and nephrotoxicity significantly limit their clinical use in kidney diseases.

miRNAs form the RNA-induced silencing complex (RISC) with AGO2 and bind primarily to the 3′ untranslated region (UTR) of target mRNAs. Unlike siRNA, miRNA typically inhibits translation through partial complementarity.[75] Artificially synthesised miRNA mimics and inhibitors are commonly employed in cell and animal studies to achieve overexpression or knockdown effects, respectively.[76] Research has demonstrated that inhibiting miR-122-5p expression and upregulating miR-4516 expression in UUO mouse models altered the expression of target genes FOXO3 and ITGA9, thereby alleviating renal injury.[87,88] A phase II clinical trial conducted by Daniel's team evaluated lademirsen, a specific miRNA-21 inhibitor intended to slow kidney function deterioration in patients with Alport syndrome. Although lademirsen was safe and well-tolerated in adult patients, there was no significant difference in eGFR compared to the placebo group, indicating that further refinements are needed for miRNA-targeted therapies to achieve clinical efficacy.[77]

Aptamers are short, single-stranded DNA or RNA molecules. They are primarily generated through a method known as systematic evolution of ligands by exponential enrichment (SELEX).[78] This method involves obtaining an oligonucleotide library with potential target-binding sequences, separating oligonucleotides that bind specifically to the target from non-binding sequences, and amplifying target-bound oligonucleotides via polymerase chain reaction (PCR).[79] The selection process is repeated multiple times to identify suitable aptamer sequences. Periostin, an ECM protein, is significantly upregulated in TGF-β-induced renal fibrosis models. Jae et al designed a periostin-binding DNA aptamer (PA) to specifically inhibit periostin expression, thereby reducing ECM deposition.[89] However, due to their inherent characteristics as single-stranded nucleic acids, aptamers are susceptible to nuclease degradation and rapid renal filtration, resulting in a short in vivo half-life ranging from minutes to hours. Additionally, despite multiple PCR-based selection rounds, off-target effects of aptamers remain relatively common. Currently, clinically approved aptamer-based drugs are limited to a few conditions, such as ocular diseases and tumours, and their application in kidney diseases warrants further investigation.

Exosomes primarily exert their therapeutic effects through miRNA delivery. In kidney diseases, expression levels of specific miRNAs are downregulated due to ischemic and hypoxic conditions. Exosomes facilitate the restoration and overexpression of these miRNAs. Additionally, different miRNAs within exosomes can bind simultaneously to the same target gene via distinct binding sites, thereby enhancing targeted suppression of specific mRNAs.[80] Moreover, multiple miRNAs may produce synergistic therapeutic effects by inhibiting various pro-fibrotic mRNAs. Encapsulation within exosomes protects miRNAs from nuclease-mediated degradation, prolonging their biological half-life. Compared with administration of miRNA mimics alone, exosomes enable more efficient cellular uptake into target cells. Furthermore, exosomes exhibit minimal toxicity and enhanced targeting capabilities, highlighting their significant therapeutic potential for treating renal fibrosis.[81]

Treatment Strategies of Exosomes from Different Sources in Renal Fibrosis

In recent years, exosomes have gained significant attention as novel carriers for intercellular communication in kidney fibrosis treatment, demonstrating great potential. They contain bioactive components, exhibit low immunogenicity, and possess targeted characteristics. Currently, Table 2 shows that exosomes derived from various sources such as bone marrow, adipose tissue, umbilical cord, and urine play important roles in kidney fibrosis treatment. Researchers are actively exploring exosomes from other tissues or cell sources, achieving notable progress in treating kidney fibrosis.

Exosomes Derived from Bone Marrow Mesenchymal Stem Cells (BMSCs-Exos)

BMSCs-Exos are small vesicular structures with therapeutic potential, showing significant effects in kidney disease treatment. CCL3, also known as macrophage inflammatory protein 1α (MIP-1α), plays a role in recruiting monocytes/macrophages and T cells in kidney diseases. Grange et al found that in a DN mouse model, exosome treatment not only downregulated MIP-1α expression, alleviating tubular injury, but also reduced Alpha-1 antitrypsin (AAT) levels in serum and urine. This effectively prevented the transformation of macrophages into fibroblasts.[91] The imbalance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) is a key factor in renal fibrosis. Almeida et al demonstrated that renal artery stenosis-induced renal vascular hypertension (RVH) activates the renin-

Table 2 Therapeutic Strategies of Exosomes in Kidney Diseases

Source	Separation Method	Disease	Model	Administration and Dose	Effects	Reference
BMSCs (Human)	UC: speed n/s, 70min	AKI	Vivo: BALB/c, IRI	Single IV dose 5×10^{10}/μg	Slowed down cell apoptosis and delayed the development of renal fibrosis	[90]
			Vitro: HK-2, oxygen–glucose deprivation	Co-culture for 24 hours		
BMSCs (Human)	UC: 100000×g, 120min	DN, CKD	Vivo: NSG, STZ	Multiple IV dose 1×10^{10} weekly for 4 weeks	Prevent macrophages from transforming into fibroblasts	[91]
BMSCs (Wistar)	UC: speed and time n/s	RVH	Vivo: Wistar, 2K1C	Single IA dose 1×10^{6}/μg	Regulated the balance of MMP-2/-9 and TIMP-1/2 expression, reducing renal inflammation and fibrosis	[92]
BMSCs (C57BL/6)	UC: 10000×g, 20min; 100000×g, 360min	RIF	Vivo: C57BL/6, UUO, IRI	Single IV dose 1×10^{6}/μg	Exosomes modified miR-34c-5p via the CD81-EGFR complex to enhance cell activation and renal interstitial fibrosis	[93]
			Vitro: pericytes, TGF-β	Co-culture for 48 hours		
BMSCs (C57BL/6)	UC: 10000×g, 30min; 100000×g, 70min	CKD	Vivo: C57BL/6, UUO	Single IV dose 100μg	Alleviated glycolysis in TECs and renal fibrosis	[94]
			Vitro: TCMK-1, glycolysis	Co-culture time n/s		
BMSCs (C57BL/6)	UC: 10000×g, 140min	CKD	Vivo: C57BL/6J, 5/6 nephrectomy	Multiple IV dose 20 μg every 2 weeks for 12 weeks	Inhibited high-PI-induced aortic calcification, improving renal and vascular function	[95]
			Vitro: VSMCs, 2.5 mM ionic phosphate	Co-culture time n/s		
AMSCs (Human)	UC: 10000×g, 30min; 100000×g, 140min	AKI	Vivo: C57BL/6J, CLP-Sepsis	Single IV dose 100μg	Promoted autophagy to reduce sepsis-induced inflammation and restore kidney function	[96]
			Vitro: HK-2, LPS	Co-culture for 24 hours		
AMSCs (Human)	UC: 10000×g, 100000×g, time n/s	AKI	Vivo: C57BL/6N, CLP	Single IV dose 2mg/kg	Alleviated kidney injury induced by sepsis, reducing vascular leakage and inflammatory factor expression	[97]
AMSCs (Human)	UC: 100000×g, 70min	RPGN	Vivo: WKY/NCrj, TF78-RPGN	Multiple IV dose $3–4\times10^{6}$/μg on day 0, day 2, day 4	Increased Tregs and M2 macrophages in the kidney, regulating the renal immune environment	[98]
			Vitro: $CD4^{+}$ T cell, M2 macrophage	Co-culture time n/s		
AMSCs (Pig)	UC: speed and time n/s	RVD	Vivo: Pig, ElRAS	Single IA dose 1×10^{11}/μg	Promoted repair and regeneration of kidney microvessels, reducing inflammation	[99]
			Vitro: LLC-PK1	Co-culture for 24 hours		

(*Continued*)

Table 2 (Continued).

Source	Separation Method	Disease	Model	Administration and Dose	Effects	Reference
AMSCs (C57BL/KsJ)	UC: 12000×g, 30min; 100000×g, 120min	DN, CKD	Vivo: C57BL/KsJ-db/db, DN	IV dose n/s	Inhibited podocyte damage in a high-glucose environment, slowing renal fibrosis	[100]
			Vitro: MP5, HG	Co-culture time n/s		
AMSCs (Human)	UC: 10000×g, 60min	DN, CKD	Vivo: SD, STZ	Multiple IV dose 50 μg twice weekly for 3 weeks	Inhibited HDAC1 and ET-1, alleviating DN symptoms and kidney damage	[101]
			Vitro: GMCs, HG	Co-culture time n/s		
Preadipocyte (Swiss)	UC: 10000×g, 20min; 120000×g, 120min	DN, CKD	Vitro: TCMK-1, IRI	Co-culture for 24 hours	Inhibited apoptosis in TCMK-1 cells, regulated the cell cycle, and improved glucose and lipid metabolism	[102]
UCMSCs (Wistar)	UC: speed and time n/s	AKI	Vitro: Wistar PTECs, gentamicin	Co-culture for 24–72 hours	Increased PTECs proliferation and DNA repair, reducing toxic damage	[103]
UCMSCs (Human)	Isolation kit	AKI	Vitro: HEK-293, IRI	Co-culture for 24–72 hours	Improved survival of damaged renal cells, reduced apoptosis and ROS accumulation, and promoted cell migration	[104]
UCMSCs (Human)	UC: 10000×g, 30min; 100000×g, 140min	AKI, CKD	Vivo: C57BL/6J, UUO	Multiple IV dose 100μg on day 1, day 3	Maintained mitochondrial function homeostasis and alleviated RTECs damage	[105]
			Vitro: HK-2, cisplatin	Co-culture for 24 hours		
UCMSCs (Human)	UC: 10000×g, 30min; 1000000×g, 70min	CKD	Vitro: PTECs, γ-irradiation-senescence	Co-culture for 72 hours	Promoted proliferation of renal tubular epithelial cells, reduced senescence, and renal fibrosis	[106]
UCMSCs (Human)	UC: 10000×g, 60min	CKD	Vivo: SD, ischemia-PN	Single IV dose 100μg	Promoted M2 macrophage polarization, improving unilateral renal ischemia and renal fibrosis	[107]
			Vitro: THP-1, LPS	Co-culture for 24 hours		
UCMSCs (Fisher 344)	Isolation kit	CKD	Vivo: Fisher 344, UUO	IV dose n/s	Alleviated PTECs EMT transformation and inhibited renal fibrosis	[108]
			Vitro: HK-2, TGF-β	Co-culture time n/s		
UCMSCs (Human)	UC 10000×g, 30min; 100000×g, 180min	CKD	Vivo: SD, UUO	Multiple IV dose 10mg/kg on day 7, day 10, day 12	Reduced ECM deposition and inhibited fibrosis	[109]
			Vitro: HK-2, TGF-β	Co-culture time n/s		

(*Continued*)

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Table 2 (Continued).

Source	Separation Method	Disease	Model	Administration and Dose	Effects	Reference
USCs (Human)	Isolation kit	AKI, CKD	Vivo: SD, IRI Vitro: HK-2, H/R	IV dose n/s Co-culture time n/s	Downregulated IRAK1/NF-κB signaling, protecting cells	[110]
USCs (Human)	UC: 100000×g, 180min	AKI, CKD	Vivo: BALB/c, nu/nu, BRC Vitro: HK-2, TGF-β	Single IV dose 2×10^6/μg Co-culture for 72 hours	Reduced RTECs necrosis and apoptosis, restoring renal function	[111]
USCs (Human)	Isolation kit	AKI, CKD	Vivo: C57BL/6J, IRI Vitro: HK-2, H/R	Single IV dose 20μg Co-culture time n/s	Inhibited ACSL4-mediated ferroptosis, reversed AKI, and delayed renal fibrosis.	[112]
USCs (Human)	UC: 20000×g, 40min; 100000×g, 90min	AKI, CKD	Vivo: SD, IRI Vitro: HK-2, H/R	IV dose n/s Co-culture for 24 hours	Promoted cell proliferation, reduced cell death, and improved AKI	[113]
USCs (Human)	UC: 100000×g, 120min	DN, CKD	Vivo: SD, STZ Vitro: RAW264.7, LPS	IV dose n/s Co-culture time n/s	Promoted conversion to anti-inflammatory M2 macrophages, alleviating kidney injury in DN rats	[114]
USCs (Human)	UC: 10000×g, 30min; 100000×g, 120min	CKD	Vivo: rabbit, UUO Vitro: HK-2, TGF-β	Multiple IV dose 400μg/kg/d for 7 days Co-culture for 24 hours	Promoted cell proliferation, migration, and angiogenesis, reducing inflammation and fibrosis	[115]
AECs (Human)	UC: 150000×g, 70min	AKI	Vivo: C57BL6/J, CLP-Sepsis Vitro: HUVECs, LPS	Single IV dose 1×10^6/μg Co-culture for 24 hours	Alleviated endothelial dysfunction, inhibited inflammation, and reduced kidney pathological damage	[116]
HLSCs (Human)	UC: 100000×g, 120min	AKI, CKD	Vivo: BALB/c, IRI	Multiple IV dose 1×109/μg on day 0, day 3	Improved renal function, alleviated renal fibrosis, and regulated KIM1, LCN2 expression	[117]
PMSCs (SD)	Isolation kit	CKD	Vivo: SD, UUO Vitro: $CD4^+$ T cell, IL-2	IV dose n/s Co-culture for 72 hours	Inhibited CD4+ T cell proliferation, inducing differentiation into Tregs and Th2 cells, reducing renal interstitial inflammation and fibrosis	[118]
BrMSCs (Human)	Isolation kit	CKD	Vivo: SD, Adenine	Multiple IV 75 μg on day 0 and day 7	Inhibited EMT and reduced ECM deposition in renal tissue	[119]

(*Continued*)

Table 2 (Continued).

Source	Separation Method	Disease	Model	Administration and Dose	Effects	Reference
IECs (Wistar)	UC: 10000×g, 80min	CKD	Vivo: Wistar, Adenine	IV dose n/s	NDK may promote the release of gut-origin exosomes and improve microvascular function in CKD rats	[120]
			Vitro: Caco-2, LPS	Co-culture time n/s		

Abbreviations: AKI, acute kidney injury; AECs, amniotic epithelial cells; AMSC, adipose mesenchymal stem cells; BMSCs, bone marrow mesenchymal stem cells; BRC, bilateral renal clamp; BrMSCs, breast milk mesenchymal stem cells; CKD, chronic kidney disease; CLP, cecal ligation and puncture; DN, diabetic nephropathy; EIRAS, experimental ischemia-reperfusion and atherosclerosis; GMCs, glomerular mesangial cells; H/R, hypoxia-reoxygenation; HK-2, human kidney-2 cell line; HUVECs, human umbilical vein endothelial cells; IA, intra-arterial injection; IECs, intestinal epithelial cells; IRI, ischemia-reperfusion injury; IV, intravenous; LPS, lipopolysaccharide; LSCs, liver stem cells; NDK, Niaodukang; n/s, not specified; PMSCs, placental mesenchymal stem cells; PN, partial nephrectomy; PTECs, proximal tubular epithelial cells; RIF, renal interstitial fibrosis; RPGN, rapidly progressive glomerulonephritis; RVD, renal vascular disease; STZ, streptozotocin; UC, ultracentrifugation; UCMSCs, umbilical cord mesenchymal stem cells; USCs, urine stem cells; UUO, unilateral ureteral obstruction; VSMCs, vascular smooth muscle cells.

angiotensin-aldosterone system (RAAS), leading to increased oxidative stress and inflammation. BMSCs-Exos help maintain vascular stability by reducing MMP-2 and MMP-9 levels, increasing TIMP-1 and TIMP-2, and enhancing IL-10 expression. This promotes the restoration of glomerular basement membrane (GBM) morphology and slows renal fibrosis progression.[92] SEMA3A, a member of the class 3 semaphorin family, participates in glomerular filtration barrier formation and regulates ureteric bud branching and podocyte differentiation. In pathological conditions, SEMA3A overexpression exacerbates kidney damage in models of AKI or CKD by inducing apoptosis and inflammation. The miR-199a-3p enriched in BMSCs-Exos targets and regulates SEMA3A expression in an ischemia-reperfusion injury (IRI) mouse model, restoring kidney function by activating AKT and ERK pathways, inhibiting apoptosis in HK-2 cells.[90] Hu et al found that BMSCs-Exos transport miR-34c-5p to pericytes, fibroblasts, and macrophages as a CD81-EGFR complex. By targeting α1,6-fucosyltransferase (FUT8), they suppress core fucosylation of proteins in these cells, inhibiting pericyte activation, fibroblast activation, and the EMT process in a renal fibrosis model.[93] Xu et al showed that miR-21a-5p in BMSCs-Exos reduces lactic acid concentration in UUO mouse renal tissue by targeting Phosphofructokinase, Muscle-type (PFKM). This downregulates glycolytic enzymes like Hexokinase 2 (HK2) and Fructose-2,6- bisphosphatase 1 (PFKFB1), delaying glycolysis induced by TGF-β in TCMK-1 cells and improving renal fibrosis by reducing the extracellular acidification rate (ECAR).[94] High mobility group box 1 (HMGB1) is a nuclear protein that promotes vascular calcification and exacerbates kidney damage in CKD by translocating from the nucleus to the cytoplasm, interacting with transcription factors, histones, and nucleosomes. This process is associated with the sirtuin protein family (SIRT1-7). Wei et al confirmed that in CKD mice, SIRT6 delivered by BMSCs-exos promotes HMGB1 deacetylation by binding to it, inhibiting HMGB1 translocation and improving renal function.[95]

Exosomes Derived from Adipose Stem Cells (ADSCs-Exos)

ADSCs-Exos are abundant, easy to obtain, and possess strong self-renewal and differentiation capabilities. In renal artery stenosis, microvessel loss in the renal cortex is primarily due to pathological changes induced by reactive oxygen species (ROS). These changes include increased vascular tone, enhanced sensitivity to vasoconstrictors, and endothelial dysfunction. ADSCs-Exos contain key genes and proteins, such as the antioxidant proteins SOD-1, catalase (CAT), and the peroxiredoxin family, which possess strong antioxidant capabilities. They alleviate systemic and renal oxidative stress, reducing the production of circulating isoprostanes (iPs) and renal superoxide anions.[121] TLR4 and TLR9 are key members of the Toll-like receptor family. TLR4 binds extensively to advanced glycation end products (AGEs) under hyperglycemic conditions, activating downstream signaling pathways and triggering oxidative stress. TLR9 is activated by endogenous ligands during renal ischemia-reperfusion injury. It promotes transcription factor activation, such as NF-κB, through MyD88-dependent or independent pathways, leading to inflammation and apoptosis in renal cells. Research shows that exosomes derived from adipose mesenchymal stem cells (AMSCs-Exos) carry miR-26a-5p and miR-342-5p, which target TLR4 and TLR9, respectively, slowing the progression of DN and AKI towards renal fibrosis.[96,100] Recent

studies have shown that exosomes can deliver circRNAs to exert protective effects. Cao et al discovered that mmu_circ_0001295 is significantly enriched in exosomes after hypoxic treatment. It protects kidneys in septic mice by downregulating pro-inflammatory factors such as IL-6 and TNF-α.[97] Yuko et al indicated that exosomes mediate macrophage regulation and influence the spleen-kidney immune network in a rapidly progressive glomerulonephritis rat model. They found that AMSCs-Exos primarily accumulate in M2 macrophages in the spleen, inducing their polarization to an anti-inflammatory phenotype. Activated M2 macrophages promote Treg proliferation. In vitro experiments showed that exosomes can independently induce Treg production when co-cultured with purified CD4+ T cells.[98] Ferguson et al found that a single renal injection of exosomes provided better protection for the microvasculature of the stenotic kidney than renal vascular reconstruction surgery. This effect may be related to the vascular protective genes carried by the exosomes. AMSCs-Exos are rich in pro-angiogenic genes such as HGF and KDR (VEGF receptors) and contain angiopoietin and members of the NOTCH signaling pathway. These components promote angiogenesis and maintain microvascular stability, improving renal blood flow and eGFR in the short term.[99] Hao et al found that miR-125a is significantly enriched in AMSCs-Exos. In DN rats, miR-125a targets and binds to HDAC1, inhibiting ET1 expression and alleviating renal fibrosis. In vitro, miR-125a overexpression increased the proliferation of rat glomerular mesangial cells through the HDAC1/ET1 axis and reduced the expression of fibrosis markers, including collagen I (Col. I) and fibronectin (FN).[101] Xu et al discovered that the traditional Chinese medicine Gan-song Yin inhibits 3T3-L1 cell proliferation, enhances glucose consumption, and reduces triglyceride levels. At the molecular level, it regulates PPARγ, GLUT4, and FABP4 protein and mRNA expression, promoting lipolysis and glucose-lipid metabolism. This reduces fat accumulation and improves cellular insulin sensitivity, alleviating the damaging effects of abnormal glucose-lipid metabolism on RTECs. Meanwhile, gansongyin (GSY) induces preadipocyte-derived exosomes to deliver miR-21-5p to regulate the TGF-β/SMAD pathway in RTECs, alleviating damage to RTECs in insulin-resistant mice.[102]

Exosomes Derived from Umbilical Cord Mesenchymal Stem Cells (UCMSCs-Exos)

UCMSCs-Exos have gained significant attention due to their remarkable effects in reducing cellular senescence, promoting kidney repair, and combating fibrosis. In kidney diseases, the accumulation of senescent cells can lead to severe issues, such as AKI, renal function decline, and kidney fibrosis. miRNA-126, one of the most abundant miRNAs in UCMSCs-Exos, plays a dual role in nephrotoxic injury. Convento et al suggest that its overexpression provides renal protective effects, while its inhibition promotes cell proliferation and DNA repair. miRNA-126 also targets Tsc1, an upstream regulatory factor of the TLR signaling pathway. It inhibits the mTOR pathway, reduces cell death, and promotes kidney regeneration.[103] Under hypoxic conditions, exosomes derived from Wharton's jelly mesenchymal stem cells (WJ-MSCs) deliver miR-210. This alleviates apoptosis and ROS accumulation in renal cells under ischemic and hypoxic conditions, enhancing cell migration and survival rates.[104] Receptor-interacting protein kinase 1 (RIPK1) is a key gene in necroptosis. It transmits necrotic signals by regulating phosphoglycerate mutase 5 (PGAM5) and promoting the dephosphorylation of dynamin-related protein 1 (Drp1), which regulates mitochondrial fission. Yu et al found that UCMSCs-Exos target and inhibit PGAM5 expression by carrying miR-874-3p. This promotes Drp1 dephosphorylation at the S637 site, maintains mitochondrial functional homeostasis, and inhibits mitochondrial fission, thereby suppressing necrosis after RTEC injury and promoting repair.[105] Additionally, Liao et al discovered that UCMSCs-Exos upregulate Lmnb1 expression in RTECs, enhancing cell proliferation activity. They also reduce the expression of the intracellular DNA break damage marker γ-H2AX and senescence markers Cdkn2a and Cdkn2d, effectively alleviating radiation-induced damage in tubular epithelial cells.[106] UCMSCs-Exos can also promote M2 macrophage polarization by transferring hepatocyte growth factor (HGF), alleviating renal fibrosis after partial nephrectomy injury.[107] Wang et al found that miR-294 and miR-133 carried by UCMSCs-Exos reduce ECM deposition and slow the EMT process and kidney injury by inhibiting TGF-β-mediated phosphorylation of SMAD2/3 and ERK1/2, respectively.[108] A disintegrin and metalloproteinase 17 (ADAM17) promotes fibrosis by activating the Notch1 protein and downstream pathways. In Shi et al's study, miR-13474 carried by exosomes targets ADAM17 and exerts therapeutic effects by inhibiting Notch1 activation.[109]

Exosomes Derived from Urinary Stem Cells (USCs-Exos)

USCs are a recently discovered type of stem cell isolated from urine. They exhibit characteristics of mesenchymal stem cells and can be easily and non-invasively obtained at a low cost. USCs also have the ability to secrete exosomes. Lu et al collected USC-derived exosomes from clinical patients for high-throughput miRNA sequencing and confirmed in a rabbit UUO model that miR-122-5p is upregulated in exosomes. This miRNA targets and inhibits SOX2 expression, exerting a therapeutic effect by influencing the AKT and ERK pathways. This results in significant improvement in renal pelvis morphology and a reduction in inflammatory infiltration and fibrosis after treatment.[115] In addition, miR-146a-5p in exosomes inhibits the activation of the NF-κB signaling pathway and the infiltration of inflammatory cells by targeting interleukin-1 receptor-associated kinase 1 (IRAK1), thereby alleviating kidney injury induced by IRI.[110] Klotho, initially identified as a tissue regeneration gene, is expressed at higher levels in the kidneys under normal conditions and plays a protective role. Studies show that after acute or chronic injury, the expression of Klotho significantly decreases in blood, urine, and kidney tissues. In both mouse AKI models and in vitro experiments, USC-Exos have been shown to upregulate Klotho protein expression by inhibiting Klotho promoter methylation, thereby suppressing kidney fibrosis progression.[111] Serine and arginine-rich splicing factor 1 (SRSF1) is a splicing regulator in the SR protein family, involved in various mRNA processes such as splicing and translation. Its overexpression inhibits apoptosis in various cells. Long-chain acyl-CoA synthetase member 4 (ACSL4) promotes ferroptosis. Research by Sun et al shows that LncRNA TUG1 regulates ACSL4 mRNA stability by interacting with SRSF1. This inhibits ferroptosis in kidney tissues of AKI mice and in HK-2 cells subjected to hypoxia-reoxygenation (H/R), exerting a therapeutic effect.[112] Recent studies have also found that circRNAs can function as carriers in exosomes. Sun et al discovered that circRNA ATG7 in USC-Exos regulates the SOCS1/STAT3 pathway by competitively binding to miR-4500.[114] This promotes M2 macrophage polarization and alleviates kidney damage in a DN rat model. Additionally, circRNA DENND4C in USC-Exos can bind to miR-138-5p, upregulating FOXO3a expression in the IRI model, promoting cell proliferation, and inhibiting NLRP3 activation. This reduces pyroptosis and slows AKI progression.[113]

Exosomes from Other Sources

In the exploration of new treatment strategies for renal fibrosis, exosomes from other sources have also been found to play a role. Recent studies show that perinatal cells, such as human amniotic epithelial cells (HAECs), can differentiate into mesodermal cells and secrete exosomes. In sepsis-induced kidney injury, the exosomes secreted by these cells inhibit the phosphorylation of P65 and activation of the NF-κB pathway. This reduces the expression of interleukins and TNF, providing protective effects on vascular endothelial cells.[116] Exosomes derived from placental mesenchymal stem cells (PMSCs-Exos) are crucial in immune regulation during kidney diseases. In the UUO model, the PMSCs-Exos treatment group showed increased infiltration of Foxp3+ cells. Seven days after UUO modeling, CD4+CD25+FOXP3+ Treg cell levels were upregulated, while IFN-γ and IL-17 expression significantly decreased. The anti-inflammatory factor IL-10 was upregulated, exerting a protective effect on the kidneys.[118] Breast milk-derived mesenchymal stem cells (BrMSCs) have high plasticity, regenerative ability, and survival capacity. The exosomes derived from them exhibit higher expression levels of anti-fibrotic miRNAs such as miR-181, miR-29b, and Let-7b. These exosomes exert anti-fibrotic effects in adenine-induced kidney injury by regulating the miR-34a/SNHG-7/AMPK/ULK-1-AKT/mTOR pathway and the expression of Beclin-1, LC3-II, and P62 mRNA and proteins.[119] In renal IRI model, exosomes derived from human liver stem cells (HLSCs) inhibit the EMT process and alleviate kidney damage by downregulating AKI-specific markers, such as KIM1 and E-selectin (SELE).[117] Niaodukang mix promotes the generation of gut-derived exosomes and releases miR-146a. This inhibits the inflammatory response in CKD rats, effectively delaying the progression of kidney fibrosis.[120]

Engineered Exosomes

In scientific research and clinical treatment, despite the availability of sufficient and effective exosomes, challenges such as low receptor cell absorption rates and weak targeting capabilities persist.[122,123] To address these limitations, researchers are focused on modifying the surface structure and composition of exosomes or loading specific cargo to

enhance their functionality, a process known as engineered exosomes. The therapeutic effect primarily involves enhancing exosomal targeting to damaged tissues and modifying the types and quantities of their cargo. Exosome engineering is mainly divided into endogenous loading and exogenous loading. Endogenous loading involves genetically modifying parent cells to integrate therapeutic drug molecules directly into exosomes, which are then produced by the cells.[124] Exogenous loading refers to the process of incorporating small cargo, including miRNAs and low molecular weight chemicals, into pre-isolated exosomes. The implementation of these strategies relies on genetic engineering, chemical modification, and physical techniques (Table 3).[125]

Table 3 Effects of Engineered Exosomes in Kidney Disease Models

Source	Disease	Model	Approach	Identification	Effects	Reference
AMSCs (Human)	AKI	Vivo: BALB/c, cisplatin Vitro: HK-2, cisplatin	Metabolic glycoengineering-mediated click chemistry	NMR spectroscopy	Specifically bind to the overexpressed CD44 in AKI and target the damaged kidneys	[126]
PMSCs (Human)	AKI	Vivo: C57BL/6, IRI Vitro: HK-2, H/R, RAW263.7, LPS+IFN-γ	Hydrogels	Rheology tests	RGD peptides enhanced exosome stability and target cell uptake efficiency, and alleviated kidney injury by inhibiting CASP3	[127]
RBCs (C57BL/6J)	AKI, CKD	Vivo: C57BL/6J, IRI, UUO Vitro: HEK293, TECs, n/s	Phage	Western blot	Engineered exosomes can reduce inflammation and fibrosis by targeting Kim-1 to accumulate in damaged renal tubules and deliver siRNAs of transcription factors P65 and Snai1	[128]
Satellite cells (C57BL/6J)	CKD	Vivo: C57BL/6J, UUO	Adenovirus	n/s	miR-29 restored the decrease in the mass of the soleus, tibialis anterior, and EDL muscles induced by UUO and alleviated kidney fibrosis by targeting TGF-β3	[129]
BMSCs (Human)	n/s	Vitro: HEK293, normal	Freeze-thaw and direct mixing	Fluorescence microscopy	Hybrid exosomes have higher transfection efficiency	[130]
UCMSCs (Human)	AKI	Vivo: ICR, cisplatin Vitro: NRK52E, cisplatin	Sonication	Fluorescence resonance energy transfer	Enhanced the uptake rate and targeting of exosomes, promoted the proliferative activity of NRK52E cells, and alleviated renal oxidation and inflammation	[131]

(*Continued*)

Table 3 (Continued).

Source	Disease	Model	Approach	Identification	Effects	Reference
AMSCs (Human)	CKD	Vivo: Nu/nu, UUO Vitro: HUVECs, hypoxia condition	Lentivirus vector	Fluorescence microscopy	Activated the PI3K/Akt/eNOS signaling pathway, relieved renal hypoxia and oxidative stress, inhibited EndoMT, and reduced renal fibrosis	[132]
KMSCs (FVB/N)	CKD	Vivo: FVB/N, renal anaemia Vitro: MDCK, PMC, HK-2, normal	Lentiviral vector	Fluorescence microscopy and qRT-PCR	Increased hemoglobin levels in CKD mice and downregulated the infiltration of F4/80-positive macrophages	[133]
BMSCs (Human)	AKI	Vivo: SCID, glycerol Vitro: TECs, H/R	Electroporation	QRT-PCR	Reduced the amount of exosomes required for treatment, alleviated tubular necrosis and hyaline tubular formation	[134]
UCMSCs (Human)	DN, CKD	Vivo: C57BL/6, STZ	Electroporation	UV–spectrophotometry	Induced CD4+Treg cells to regulate intestinal microbiota metabolism to reduce kidney injury	[135]
Orange	IgAN	Vivo: BALB/c, SEB, BSA Vitro: PPs, ConA, IL-2	Electroporation	UV-spectrophotometry	Reduced proteinuria, alleviated mesangial hyperplasia and IgA deposition, and decreased the percentage of LIGHT +CD4+ cells	[136]
ToMCSs (Human)	CKD	Vivo: ICR, 5/6 nephrectomy	CRISPR-Cas9 system	Thermogravimetric analysis	Engineered exosomes promote stem cell migration and tubule and blood vessel generation by targeting CXCR4	[137]

Abbreviations: ConA, concanavalin A; EDL, extensor digitorum longus; EndoMT, endothelial-to-mesenchymal transition; eNOS, endothelial nitric oxide synthase; ERCs, endometrial regenerative cells; IgAN, immunoglobulin A nephropathy; KMSCs, kidney mesenchymal stem cells; LTH, linear tubular homing peptide; NMR, nuclear magnetic resonance; PMC, peritoneal mesothelial cells; PPs, peyer's patches; qRT-PCR, quantitative real-time polymerase chain reaction; RBCs, red blood cells; R-Exos, RBCs-exosomes; RGD, arginine-glycine-aspartic acid peptide; SEB, staphylococcal enterotoxin B; ToMCSs, tonsil mesenchymal stem cells; UV-Vis, ultraviolet-visible spectrophotometry.

Enhanced Targeting of Exosomes

Numerous methods, including physical methods, chemical modifications, and genetic engineering, have been developed for exosome engineering. Chemical modification methods are predominantly utilised to enhance exosomal targeting. Covalent modification involves attaching modified molecules to exosomes via chemical bonds, thereby improving recognition and binding to target tissues. One research group introduced azide groups ($-N_3$) onto the surface of human AMSCs using metabolic labeling. They coupled PEGylated hyaluronic acid (P-HA) to the cell surface through bioorthogonal click chemistry. Exosomes with surface-modified P-HA were then isolated from the cell culture supernatant. The P-HA on the exosomal surface can specifically bind to CD44, which is overexpressed at the site of AKI, and

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TLR4 on the surface of M1 macrophages, enabling precise localisation to damaged kidneys. Engineered exosomes demonstrated kidney accumulation 2.56-fold greater than the control group, and the kidney-to-liver signal ratio was significantly higher (1.04 vs 0.73), substantially enhancing their targeting efficiency to injured kidneys.[126] Zhang et al assembled biotinylated arginine-glycine-aspartic acid (RGD) peptides into hydrogels and improved the binding ability of RGD to integrins on the exosome membrane through chemical coupling. This not only increased the stability and targeting of the exosomes but also extended their residence time at the site of kidney injury, thereby more effectively promoting kidney repair, anti-fibrosis, anti-apoptosis, and autophagy regulation.[127] Tang et al effectively delivered P65 and Snai1 siRNA to damaged RTECs using red blood cell-derived exosomes (R-Exos) modified with the targeting peptide LTH, which binds to kidney injury molecule-1 (Kim-1). This dual inhibition significantly reduced P-p65 and Snai1 expression, thereby suppressing renal inflammation and fibrosis in IRI and UUO models, while attenuating the progression of ischemic AKI to CKD.[128] Wang et al transfected the vector containing the exosomal membrane protein gene Lamp2b, combined with the rabies virus glycoprotein targeting peptide (RVG-TP), into mouse satellite cells. They then used an adenovirus containing miR-29ab1 and miR-29b2c for transduction, enabling the satellite cells to secrete exosomes rich in miR-29, which may accumulate in the kidneys and potentially alleviate renal fibrosis through the TGF-β signaling pathway.[129] In recent years, methods for generating hybrid exosomes have continuously evolved. Gharehchelou et al hybridised BMSCs-Exos with liposomes using freeze-thaw and direct mixing methods, significantly enhancing the uptake efficiency of the resulting hybrids by HEK293T cells.[130] And exosomes from different sources can also fuse with each other to exert a synergistic effect, Wu et al constructed engineered hybrid exosomes fused with nanovesicles derived from human neutrophil membranes and hucMSC-Exos, named neutrophil membrane engineered hucMSC-Exos (NEX).[131]

Improvement in Drug Delivery Efficiency

Exosomes, as natural carriers, possess strong penetration capabilities and superior delivery efficiency compared to liposomes and other synthetic drug-delivery systems. Recent research has increasingly focused on utilising exosomes as targeted drug carriers.[138] Li et al introduced the glial cell-derived neurotrophic factor (GDNF) gene into MSCs to obtain engineered GDNF-AMSCs-Exos. These exosomes improve renal microcirculation through the PI3K/Akt/eNOS signaling pathway, reducing renal hypoxia and oxidative stress, while inhibiting endothelial-mesenchymal transition (EndoMT).[132] Choi et al inserted the cDNA of human EPO into a lentiviral vector co-expressing green fluorescent protein (GFP) to construct a plasmid expressing EPO mRNA. By co-transfecting HEK293T cells with the packaging construct, they produced lentiviral supernatant. Exosomes derived from kidney-derived mesenchymal stem cells (KMSCs) transduced with this supernatant improved hemoglobin levels and renal function in CKD mice.[133] Besides chemical modifications, electroporation is another commonly used physical engineering technique for exosomes. Electroporation creates temporary micropores in the exosomal membrane via an electric field, allowing exogenous substances or therapeutic agents to enter exosomes.[139] Studies have shown that electroporation can transfer mimics of miR-127, miR-486, miR-10a, and miR-29a into MSCs, resulting in high expression of these miRNAs in the stem cells and their secreted exosomes. In an in vivo model of AKI, this treatment promotes kidney regeneration and slows the progression of kidney fibrosis.[134] Wang et al successfully loaded Exendin-4 (Ex-4), a glucagon-like peptide-1 (GLP-1) receptor agonist, into HUCMSCs-Exos using innovative electroporation technology, creating the HUCMSCs-Exo@Ex-4 complex. This complex regulates gut microbiota metabolism and induces CD4+ Treg cell production, reducing inflammatory responses and effectively inhibiting renal fibrosis in a DN mouse model.[135] Zhang et al isolated exosomes from orange juice and used electroporation to load dexamethasone sodium phosphate (DexP), which inhibits lymphocyte activation, reduces proteinuria, and alleviates renal pathological damage in an IgA nephropathy (IgAN) mouse model.[136] Kim et al utilized a novel optogenetic exosome technology to load NF-κB super-repressor inhibitors into exosomes, reducing tubular interstitial damage by decreasing cell apoptosis and immune cell infiltration.[174] In addition, the CRISPR-Cas9 system is frequently employed for genetic engineering of exosomes. CRISPR-Cas9 technology primarily consists of clustered regularly interspaced short palindromic repeats (CRISPR), CRISPR-associated protein 9 (Cas9), and a single-stranded guide RNA (sgRNA) molecule complementary to the target DNA sequence. This system can induce a double strand break (DSB) at the target gene, achieving gene knockout through the non-homologous end-joining

(NHEJ) mechanism or facilitating gene insertion or replacement via homology-directed repair (HDR).[140] CRISPR-Cas9 systems are classified into types I, II, and III based on their reliance on the protospacer adjacent motif (PAM) sequence, with type II being the most widely used due to its targeting via a single Cas protein.[141] Lee et al utilized the CRISPR-Cas9 system to insert the SDF-1α gene into the AAVS1 locus of tonsil-derived mesenchymal stem cells (ToMSCs) to achieve overexpression. They obtained exosomes from the overexpressed ToMSCs and intermediate mesoderm cells (IMCs) differentiation, combining them to form multifunctional hybrid engineered exosomes to promote renal tissue regeneration.[137] Shao et al employed CRISPR-Cas9 technology to specifically knock down CD73 expression in exosomes derived from endometrial regenerative cells (ERC-Exos), demonstrating the essential role of CD73 in attenuating inflammatory factor expression in fibrotic kidneys via the MAPK pathway.[175]

Application of Exosomes in Clinical Practice

Exosomes are gradually transitioning to clinical research, offering novel therapeutic strategies for kidney diseases. A study by Nassar et al involved 40 patients with stage III–IV CKD randomly divided into two groups of 20. The treatment group received two doses of exosomes derived from umbilical mesenchymal stem cells. At 12 weeks, patients exhibited significant reductions in Scr, BUN, and IL-10 levels, among other indicators; however, by the end of one year, these effects were no longer statistically significant, suggesting temporary therapeutic benefits of exosome treatment.[142] In other biological systems, exosomes can also exert therapeutic effects by inhibiting inflammatory immune responses. A recent study by Azam et al provided the first confirmation of the efficacy of WJMSC-Exos for immune-mediated dry eye through a rigorous triple-blind randomised controlled trial (RCT). The study included 16 participants, with each patient's eyes randomly assigned to either the exosome intervention or the control group, administered twice daily for two weeks. Results showed a significant increase in tear secretion and a reduction in ocular discomfort in the exosome treatment group. Moreover, levels of IL-6 and MMP-9 in tear fluid were significantly reduced, providing strong evidence for exosomal roles in reducing inflammation and alleviating tissue damage.[143]

Uraemic toxin-induced vascular dysfunction is closely associated with exosomes. A study involving 30 patients with ESRD showed that, compared to bicarbonate haemodialysis (BHD), mixed online haemodiafiltration (mOL-HDF) significantly reduced the expression of pro-atherosclerotic miR-223 in plasma-derived exosomes, an effect maintained throughout the 3- to 9-month treatment period. In vitro experiments confirmed that elevated miR-223 levels in plasma exosomes from dialysis patients inhibited angiogenesis in HUVECs and exacerbated calcification in vascular smooth muscle cells (VSMCs). These findings provide a rationale for developing engineered exosomes loaded with miR-223 antagonists.[144] Recent clinical studies have confirmed that exosomes promote angiogenesis and reduce ECM deposition, particularly in epidermal wound healing and scar repair. Kwon et al conducted a 12-week double-blind RCT involving 25 patients with facial atrophic acne scars. One side of the face received carbon dioxide laser treatment combined with AMSCs-Exo gel, while the other side received control gel. Results indicated significantly greater scar improvement in the AMSCs-Exo treatment group compared to the placebo group, evidenced by reduced scar area, decreased skin roughness, and shorter duration of post-inflammatory erythema.[145]

CKD is frequently accompanied by renal ischaemia and hypoxia, leading to impaired mitochondrial oxidative phosphorylation and excessive accumulation of ROS, further exacerbating renal damage. Current in vitro studies have demonstrated that exosomes regulate the Nrf2-mediated oxidative stress pathway in the kidneys by targeting FAM129B, thereby reducing ROS accumulation.[146] ROS accumulation is also a common clinical feature implicated in Alzheimer's disease (AD). Xie et al conducted a phase I/II clinical trial to evaluate the efficacy of allogeneic AMSCs-Exos in patients with mild-to-moderate AD. This trial involved nine patients divided into low-, medium-, and high-dose groups based on exosome dosage. Treatments were administered nasally twice weekly for 12 weeks, followed by a 48-week follow-up period. Results demonstrated continued cognitive function improvement up to 36 weeks post-treatment and a slowed rate of hippocampal atrophy in the medium-dose group. These benefits may result from brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) carried by exosomes, which promote neuronal regeneration and synaptic plasticity, inhibit ROS accumulation, and thus alleviate neurodegenerative changes and cognitive decline.[147]

It is noteworthy that exosomes also show therapeutic potential in fibrotic diseases of other organ systems. Li et al confirmed that nebulised HUCMSC-Exos can alleviate pulmonary fibrosis by regulating tissue repair processes. They

randomly divided 24 patients into two groups: one received conventional treatment combined with nebulised HUCMSC-Exos twice daily for a week, while the other group received only conventional treatment. After 12 weeks, patients receiving exosome therapy demonstrated significant improvements in forced vital capacity (FVC) and maximum voluntary ventilation (MVV), without serious adverse events. Furthermore, high-resolution CT (HRCT) imaging in two patients revealed significant reductions in pulmonary fibrosis lesions, strongly supporting the therapeutic efficacy of exosomes in fibrotic tissue improvement.[148]

Problems and Challenges

Difficulties in Separation and Purification

Due to their small size, exosomes are challenging to isolate and purify efficiently from complex biological samples. Various factors also increase their heterogeneity. Recent studies indicate that the cellular microenvironment influences exosome heterogeneity, as components of the culture medium can indirectly regulate this heterogeneity by altering parent cell functionality, leading to variable immunoregulatory effects. Furthermore, exosomes exhibit size-based subpopulations, which affect their quantity and function.[149] Even exosomes derived from identical cell types display significant batch-to-batch variability, further limiting their separation and clinical application.[150] Ultracentrifugation (UC) is currently the most common method for extracting exosomes from various sources, however, this technique is cumbersome, produces low yields, and frequently co-precipitates other vesicles and protein aggregates, exacerbating product heterogeneity.[151] The immunocomplex precipitation technique (ICPT) is simple but tends to co-precipitate lipid proteins and other contaminants, making it suitable primarily for rapid screening of disease markers in urine or serum.[152] Size exclusion chromatography (SEC) provides higher purity but requires prolonged separation times and specialised equipment, making it suitable primarily for stem-cell-derived exosome production.[153] Tangential flow filtration (TFF) enhances yield and reduces clogging risk by increasing surface area through tangential flow contact with a permeable membrane; however, shear forces may damage exosome structures, making it suitable for high-throughput samples in continuous production.[154] Immunoaffinity capture technology (IACT) isolates exosomes with high specificity, but antibody binding fluctuations during large-scale applications may compromise batch consistency.[155] In recent years, microfluidic technology has demonstrated advantages in high separation efficiency, precision, and rapid medical diagnosis through miniaturisation and accurate control, serving as a key technology bridging basic research with clinical translation. However, high costs limit its application primarily to small sample volumes.[156] Differences among current isolation methods complicate the comparison of exosome characteristics, functions, and compositions. Therefore, a consensus on standardized exosome isolation procedures is essential for biological research and clinical applications.[157]

Identification of Exosomes

Current methods for exosome identification and detection face several challenges. Available techniques can be broadly classified into morphological characterisation and surface marker detection. Although traditional electron microscopy reveals exosomal morphology, it is limited in analysing their molecular composition. Exosomal surface markers mainly include HSPs and tetraspanins.[158] CD63, a member of the tetraspanin family, is highly enriched in the luminal vesicles of MVBs from various cell sources. Its large extracellular epitope allows easy antibody binding; however, CD63 is also expressed on microvesicles secreted from lysosomes, potentially causing false-positive results.[159,160] In contrast, CD9 and CD81, more widely distributed on the plasma membrane, exhibit slightly lower specificity, and their epitopes may be masked, reducing detection efficiency.[161,162] Nevertheless, CD9 and CD81 are highly expressed on exosomes from HeLa cells, MSCs, neurons, and other sources.[163] In addition, certain proteins in exosomes are present at low levels, which can hinder the sensitivity of mass spectrometry (MS) and enzyme-linked immunosorbent assay (ELISA), thereby limiting the comprehensive analysis of exosomal proteomics.[164] Flow cytometry, while useful, suffers from insufficient sensitivity when detecting smaller exosomes and typically only identifies surface markers.[165] Therefore, the optimal current approach for exosome identification involves the simultaneous detection of at least two markers among CD63, CD9, and CD81, combined with cytoplasmic proteins (eg TSG101 or Alix), and supplemented by comprehensive morphological and nanoparticle tracking analysis (NTA).[166] Nevertheless, substantial challenges remain concerning the accuracy

and reliability of these methods, complicating efforts to establish uniform standards. Consequently, there is an urgent need to develop advanced technological approaches to support in-depth studies on exosomes.

Stability and Preservation of Exosomes

Exosomes are highly sensitive to environmental factors that can affect their structure and function. Variations in conditions such as temperature, pH, and ionic strength can disrupt the exosomal membrane, leading to content leakage or functional loss.[167] Additionally, the presence of nucleases and proteases in bodily fluids or storage environments can degrade key exosomal components, reducing their utility in clinical applications.[168] Although storing exosomes in phosphate-buffered saline (PBS) at −80°C has been shown to preserve their contents, freezing may impact the stability of the exosomal lipid membrane. The formation of ice crystals during freezing can cause mechanical damage, resulting in the loss of contents and corresponding biological functions.[169] Bosch et al employed trehalose as a stabilizer in preservation solutions, replacing alginate/sucrose, which significantly reduced intracellular ice formation during freezing and prevented protein aggregation.[170] PBS supplemented with human serum albumin and trehalose (PBS-HAT) at −80°C protects exosomes by maintaining osmotic pressure, pH balance, and ionic conditions, making it suitable for multiple freeze-thaw cycles, sample dilution, and storage.[171] However, the stability of exosome samples varies depending on their source, as different exosomes possess inherent properties that affect their preservation. This variation underscores the need for standardized guidelines for exosome storage. Therefore, further research is needed to identify optimal storage conditions for exosomes from different sources, ensuring their stability and functionality.

Barriers to Clinical Adoption

Current exosome research primarily remains at the preclinical stage, with clinical translation significantly limited by immune rejection responses and efficacy uncertainties. Exosomes contain immune-related molecules such as IL-6 and TNF-α both externally and internally. Under specific conditions, these biomolecules can activate immune cells, particularly macrophages, altering their phenotype and stimulating further inflammatory mediator release.[172] Although studies have demonstrated exosomes' potential in reducing immune rejection responses, significant differences in key immune-related molecules, such as major histocompatibility complex (MHC), between donor and recipient could readily trigger unintended immune reactions, highlighting the persistent immunogenic risks of exosomes.[173] Furthermore, the in vivo distribution, metabolism, and mechanisms of action of exosomes remain incompletely understood. In drug delivery applications, uncertainties persist regarding precise lesion targeting and effective, controlled drug release. Additionally, the dose-response relationship of exosome-based therapies is not yet clearly defined.[176] These unresolved issues reflect both gaps in scientific understanding and substantial regulatory hurdles, necessitating extensive validation studies. Additionally, scalability issues such as large-scale manufacturing, standardised preparation, and quality control remain unresolved, further restricting clinical applications. Future research efforts should therefore prioritise elucidating exosomal mechanisms of action, establishing clear dosing protocols and efficacy assessment frameworks, and rigorously designing clinical trials to evaluate therapeutic efficacy and safety. Under strict adherence to good manufacturing practice (GMP), production techniques should be optimized to address scalability issues, thereby facilitating the clinical translation of exosome-based therapeutics.

Conclusion

This review underscores the multifaceted value of exosomes in renal fibrosis research, offering novel insights into this pathological process. As pivotal mediators of intercellular communication, exosomes participate in kidney development and maintain physiological homeostasis under normal conditions, while also promoting fibrosis in pathological contexts by transmitting specific nucleic acids, proteins, and other bioactive substances, thus exhibiting dual functionality. The proteins and nucleic acids encapsulated within exosomes provide precise biomarkers for the early diagnosis and evaluation of renal fibrosis. Furthermore, naturally derived and engineered exosomes exhibit promising therapeutic potential for delaying renal disease progression by targeting inflammatory responses, oxidative stress, and fibrotic signalling pathways. Moving forward, interdisciplinary collaboration will be essential in overcoming existing barriers,

positioning exosomes as innovative tools for diagnosing and treating renal fibrosis, and ultimately providing new treatment hope for patients with CKD.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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References

1. Romagnani P, Agarwal R, Chan JCN, et al. Chronic kidney disease. *Nat Rev Dis Primers*. 2025;11(1):8. doi:10.1038/s41572-024-00589-9
2. ChesnChaaye NC, Ortiz A, Zoccali C, Stel VS, Jager KJ. The impact of population ageing on the burden of chronic kidney disease. *Nat Rev Nephrol*. 2024;20(9):569–585. doi:10.1038/s41581-024-00863-9
3. Noels H, van der Vorst EPC, Rubin S, et al. Renal-cardiac crosstalk in the pathogenesis and progression of heart failure. *Circ Res*. 2025;136 (11):1306–1334. doi:10.1161/CIRCRESAHA.124.325488
4. Correa-Rotter R, Maple-Brown LJ, Sahay R, Tuttle KR, Ulasi II. New and emerging therapies for diabetic kidney disease. *Nat Rev Nephrol*. 2024;20(3):156–160. doi:10.1038/s41581-023-00782-1
5. Ndongo M, Nehemie LM, Coundoul B, Diouara AAM, Seck SM. Prevalence and outcomes of polycystic kidney disease in African populations: a systematic review. *World J Nephrol*. 2024;13(1):90402. doi:10.5527/wjn.v13.i1.90402
6. Chung JYF, Zhang YY, Ji ZZY, et al. Immunodynamics of macrophages in renal fibrosis. *Integr Med Nephrol Androl*. 2023;10(3):e00001. doi:10.1097/IMNA-D-23-00001
7. Khandpur S, Mishra P, Mishra S, Tiwari S. Challenges in predictive modelling of chronic kidney disease: a narrative review. *World J Nephrol*. 2024;13(3):97214. doi:10.5527/wjn.v13.i3.97214
8. Abbad L, Esteve E, Chatziantoniou C. Advances and challenges in kidney fibrosis therapeutics. *Nat Rev Nephrol*. 2025;21(5):314–329. doi:10.1038/s41581-025-00934-5
9. Liu Y. Kidney fibrosis: fundamental questions, challenges, and perspectives. *Integr Med Nephrol Androl*. 2024;11(4):e24–00027. doi:10.1097/IMNA-D-24-00027
10. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell*. 1983;33(3):967–978. doi:10.1016/0092-8674(83)90040-5
11. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem*. 1987;262(19):9412–9420. doi:10.1016/S0021-9258(18)48095-7
12. Arya SB, Collie SP, Parent CA. The ins-and-outs of exosome biogenesis, secretion, and internalization. *Trends Cell Biol*. 2024;34(2):90–108. doi:10.1016/j.tcb.2023.06.006
13. Ergunay T, Collino F, Bianchi G, Sedrakyan S, Perin L, Bussolati B. Extracellular vesicles in kidney development and pediatric kidney diseases. *Pediatr Nephrol*. 2024;39(7):1967–1975. doi:10.1007/s00467-023-06165-9
14. Kosanović M, Llorente A, Glamočlija S, Valdivielso JM, Bozic M. Extracellular vesicles and renal fibrosis: an odyssey toward a new therapeutic approach. *Int J Mol Sci*. 2021;22(8):3887. doi:10.3390/ijms22083887
15. Singh S, Paul D, Nath V, Ahuja R. Exosomes: current knowledge and future perspectives. *Tissue Barriers*. 2024;12(2):2232248. doi:10.1080/21688370.2023.2232248
16. Zhang Y, Bi JY, Huang JY, Tang YN, Du SY, Li PY. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. *Int J Nanomed*. 2020;15:6917–6934. doi:10.2147/IJN.S264498
17. Solinger JA, Ott DP, Spang A. ESCRTing the RABs through conversion. *Biochem Soc Trans*. 2025;53(2):431–445. doi:10.1042/BST20253007
18. Mukerjee N, Bhattacharya A, Maitra S, et al. Exosome isolation and characterization for advanced diagnostic and therapeutic applications. *Mater Today Bio*. 2025;31:101613. doi:10.1016/j.mtbio.2025.101613
19. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478):6977. doi:10.1126/science.aau6977

20. Zhang HJ, Liu XY, Shi JM, et al. Research progress on the mechanism of exosome- mediated virus infection. *Front Cell Infect Microbiol*. 2024;14:1418168. doi:10.3389/fcimb.2024.1418168
21. Xie SM, Zhang Q, Jiang L. Current knowledge on exosome biogenesis, cargo-sorting mechanism and therapeutic implications. *Membranes*. 2022;12(5):498. doi:10.3390/membranes12050498
22. Feng F, Wang LJ, Li JC, Chen TT, Liu L. Role of heparanase in ARDS through autophagy and exosome pathway (review). *Front Pharmacol*. 2023;14:1200782. doi:10.3389/fphar.2023.1200782
23. Ai Y, Guo C, Garcia-Contreras M, et al. Endocytosis blocks the vesicular secretion of exosome marker proteins. *Sci Adv*. 2024;10(19):eadi9156. doi:10.1126/sciadv.adi9156
24. Majer O, Liu B, Kreuk LSM, Krogan N, Barton GM. UNC93B1 recruits syntenin-1 to dampen TLR7 signalling and prevent autoimmunity. *Nature*. 2019;575(7782):366–370. doi:10.1038/s41586-019-1612-6
25. Zhang YF, Li YQ, Liu P, et al. Phosphatase Shp2 regulates biogenesis of small extracellular vesicles by dephosphorylating Syntenin. *J Extracell Vesicles*. 2021;10(5):e12078. doi:10.1002/jev2.12078
26. Habibi J, DeMarco VG, Hulse JL, et al. Inhibition of sphingomyelinase attenuates diet - Induced increases in aortic stiffness. *J Mol Cell Cardiol*. 2022;167:32–39. doi:10.1016/j.yjmcc.2022.03.006
27. Savio LEB, de Andrade Mello P, Santos S, et al. P2X7 receptor activation increases expression of caveolin-1 and formation of macrophage lipid rafts, thereby boosting CD39 activity. *J Cell Sci*. 2020;133(5):237560. doi:10.1242/jcs.237560
28. Choezom D, Plum JM, Karuna MP, et al. The ceramide-dependent EV secretome differentially affects prostate cancer cell migration. *Cells*. 2025;14(7):547. doi:10.3390/cells14070547
29. Albacete-Albacete L, Navarro-Lerida I, Lopez JA, et al. ECM deposition is driven by caveolin1 -dependent regulation of exosomal biogenesis and cargo sorting. *J Cell Biol*. 2020;219(11):e202006178. doi:10.1083/jcb.202006178
30. Wei D, Zhan W, Gao Y, et al. RAB31 marks and controls an ESCRT-independent exosome pathway. *Cell Res*. 2021;31(2):157–177. doi:10.1038/s41422-020-00409-1
31. Xu K, Feng H, Zhao R, Huang Y. Targeting tetraspanins at cell interfaces: functional modulation and exosome-based drug delivery for precise disease treatment. *ChemMedChem*. 2025;20(2):e202400664. doi:10.1002/cmdc.202400664
32. Lindström NO, McMahon JA, Guo J, et al. Conserved and divergent features of human and mouse kidney organogenesis. *J Am Soc Nephrol*. 2018;29(3):785–805. doi:10.1681/ASN.2017080887
33. Krause M, Rak-Raszewska A, Naillat F, et al. Exosomes as secondary inductive signals involved in kidney organogenesis. *J Extracell Vesicles*. 2018;7(1):1422675. doi:10.1080/20013078.2017.1422675
34. Shen AR, Zhong X, Tang TT, et al. Integrin, exosome and kidney disease. *Front Physiol*. 2021;11:627800. doi:10.3389/fphys.2020.627800
35. Müller-Deile J, Sopel N, Ohs A, et al. Glomerular endothelial cell-derived microRNA-192 regulates nephronectin expression in idiopathic membranous glomerulonephritis. *J Am Soc Nephrol*. 2021;32(11):2777–2794. doi:10.1681/ASN.2020121699
36. Yu MJ, Shen XG, He WF, Zheng DN, He Q, Jin J. *Mesangial cell-derived exosomal mir*-4455 induces podocyte injury in iga nephropathy by targeting ULK2. *Oxid Med Cell Longev*. 2022;2022:1740770. doi:10.1155/2022/1740770
37. Blijdorp CJ, Hartjes TA, Wei KY, et al. Nephron mass determines the excretion rate of urinary extracellular vesicles. *J Extracell Vesicles*. 2022;11(1):e12181. doi:10.1002/jev2.12181
38. Street JM, Birkhoff W, Menzies RI, et al. Exosomal transmission of functional aquaporin 2 in kidney cortical collecting duct cells. *J Physiol*. 2011;589:6119–6127. doi:10.1113/jphysiol.2011.220277
39. Itabashi Y, Ravichandran R, Bansal S, et al. Role for exosomes with self-antigens and immune regulatory molecules in allo- and auto-immunity leading to chronic immune injury following murine kidney transplantation. *Transpl Immunol*. 2022;75:101702. doi:10.1016/j.trim.2022.101702
40. Pang XL, Wang ZG, Liu L, et al. Immature dendritic cells derived exosomes promotes immune tolerance by regulating T cell differentiation in renal transplantation. *Aging*. 2019;11(20):8911–8924. doi:10.18632/aging.102346
41. Liu X, Liu Z, Wang C, et al. Kidney tubular epithelial cells control interstitial fibroblast fate by releasing TNFAIP8- encapsulated exosomes. *Cell Death Dis*. 2023;14(10):672–677. doi:10.1038/s41419-023-06209-w
42. Chen S, Zhang M, Li J, et al. β-catenin-controlled tubular cell-derived exosomes play a key role in fibroblast activation via the OPN-CD44 axis. *J Extracell Vesicles*. 2022;11(3):e12203. doi:10.1002/jev2.12203
43. Liu X, Miao J, Wang C, et al. Tubule-derived exosomes play a central role in fibroblast activation and kidney fibrosis. *Kidney Int*. 2020;97(6):1181–1195. doi:10.1016/j.kint.2019.11.026
44. Zhao S, Li W, Yu W, et al. Exosomal miR-21 from tubular cells contributes to renal fibrosis by activating fibroblasts via targeting PTEN in obstructed kidneys. *Theranostics*. 2021;11(18):8660–8673. doi:10.7150/thno.62820
45. Zhou X, Zhao S, Li W, et al. Tubular cell-derived exosomal miR-150-5p contributes to renal fibrosis following unilateral ischemia-reperfusion injury by activating fibroblast in vitro and in vivo. *Int J Biol Sci*. 2021;17(14):4021–4033. doi:10.7150/ijbs.62478
46. Lv LL, Feng Y, Wu M, et al. Exosomal miRNA-19b-3p of tubular epithelial cells promotes M1 macrophage activation in kidney injury. *Cell Death Differ*. 2020;27(1):210–226. doi:10.1038/s41418-019-0349-y
47. Zapała B, Kamińska A, Piwowar M, Paziewska A, Gala-Błądzińska A, Stępień EŁ. miRNA signature of urine extracellular vesicles shows the involvement of inflammatory and apoptotic processes in diabetic chronic kidney disease. *Pharm Res*. 2023;40(4):817–832. doi:10.1007/s11095-023-03481-5
48. Wu X, Gao Y, Xu L, et al. Exosomes from high glucose-treated glomerular endothelial cells trigger the epithelial-mesenchymal transition and dysfunction of podocytes. *Sci Rep*. 2017;7(1):9371. doi:10.1038/s41598-017-09907-6
49. Zhu QJ, Zhu M, Xu XX, Meng XM, Wu YG. Exosomes from high glucose-treated macrophages activate glomerular mesangial cells via TGFbeta1/Smad3 pathway in vivo and in vitro. *FASEB J*. 2019;33:9279–9290. doi:10.1096/fj.201802427RRR
50. Chai F, Chang X, Lin Y, et al. Effect of M0 macrophage-derived exosome miR-181d-5p targeting BCL-2 to regulate NLRP3/caspase-1/GSDMD pathway on human renal mesangial cells pyroptosis. *Gene*. 2024;908:148289. doi:10.1016/j.gene.2024.148289
51. Yu W, Song J, Chen S, et al. Myofibroblast-derived exosomes enhance macrophages to myofibroblasts transition and kidney fibrosis. *Ren Fail*. 2024;46(1):2334406. doi:10.1080/0886022X.2024.2334406
52. Fujii N, Urabe F, Yamamoto S, Inoue K, Kimura T, Shiraishi K. Extracellular vesicles in renal cell carcinoma: a review of the current landscape and future directions. *Urol Oncol*. 2025;43(6):370–379. doi:10.1016/j.urolonc.2025.02.022

53. Huda MN, Nafiujjaman M, Deaguero IG, et al. Potential use of exosomes as diagnostic biomarkers and in targeted drug delivery: progress in clinical and preclinical applications. *ACS Biomater Sci Eng*. 2021;7(6):2106–2149. doi:10.1021/acsbiomaterials.1c00217
54. Gudehithlu KP, Hart P, Joshi A, et al. Urine exosomal ceruloplasmin: a potential early biomarker of underlying kidney disease. *Clin Exp Nephrol*. 2019;23(8):1013–1021. doi:10.1007/s10157-019-01734-5
55. Lv LL, Cao YH, Pan MM, et al. CD2AP mRNA in urinary exosome as biomarker of kidney disease. *Clin Chim Acta*. 2014;428:26–31. doi:10.1016/j.cca.2013.10.003
56. Zhao Z, Yan Q, Fang L, et al. Identification of urinary extracellular vesicles differentially expressed RNAs in diabetic nephropathy via whole-transcriptome integrated analysis. *Comput Biol Med*. 2023;166:107480. doi:10.1016/j.compbiomed.2023.107480
57. Bai X, Geng J, Li X, et al. Long noncoding RNA LINC01619 regulates MicroRNA-27a/Forkhead box protein O1 and endoplasmic reticulum stress-mediated podocyte injury in diabetic nephropathy. *Antioxid Redox Signal*. 2018;29(4):355–376. doi:10.1089/ars.2017.7278
58. Cao Y, Shi Y, Wang Y, et al. Exosomal hsa_circ_0008925 from urine is related to chronic renal fibrosis. *Dis Markers*. 2022;2022(1):1899282. doi:10.1155/2022/1899282
59. Cao Y, Shi Y, Yang Y, et al. Urinary exosomes derived circRNAs as biomarkers for chronic renal fibrosis. *Ann Med*. 2022;54(1):1966–1976. doi:10.1080/07853890.2022.2098374
60. Lv LL, Cao YH, Ni HF, et al. MicroRNA-29c in urinary exosome/microvesicle as a biomarker of renal fibrosis. *Am J Physiol Renal Physiol*. 2013;305(8):1220–1227. doi:10.1152/ajprenal.00148.2013
61. Yu Y, Bai F, Qin N, et al. Non-proximal renal tubule-derived urinary exosomal miR-200b as a biomarker of renal fibrosis. *Nephron*. 2018;139(3):269–282. doi:10.1159/000487104
62. Yamamoto CM, Murakami T, Oakes ML, et al. Uromodulin mRNA from urinary extracellular vesicles correlate to kidney function decline in type 2 diabetes mellitus. *Am J Nephrol*. 2018;47(5):283–291. doi:10.1159/000489129
63. Alves Dos santos K, Clemente Dos Santos IC, Santos Silva C, Gomes Ribeiro H, de Farias Domingos I, Nogueira Silbiger V. Circulating exosomal miRNAs as biomarkers for the diagnosis and prognosis of colorectal cancer. *Int J Mol Sci*. 2020;22(1):346–352. doi:10.3390/ijms22010346
64. Flores-Chova A, Martinez-Arroyo O, Riffo-Campos AL, Ortega A, Forner MJ, Cortes R. Plasma exosomal non-coding RNA profile associated with renal damage reveals potential therapeutic targets in lupus nephritis. *Int J Mol Sci*. 2023;24(8):7088. doi:10.3390/ijms24087088
65. Yang D, Yin R, Zhang X, et al. Circulating exosome-circRNAs mediated downregulation of FGF9 through ceRNA mechanism aggravates renal fibrosis in diabetic nephropathy. *PLoS One*. 2025;20(6):e0326217. doi:10.1371/journal.pone.0326217
66. Saejong S, Townamchai N, Somparn P, et al. MicroRNA-21 in plasma exosome, but not from whole plasma, as a biomarker for the severe interstitial fibrosis and tubular atrophy (IF/TA) in post-renal transplantation. *Asian Pac J Allergy Immunol*. 2022;40(1):94–102. doi:10.12932/AP-101019-0656
67. Chen F, Shi B, Liu W, et al. Circulating exosomal microRNAs as biomarkers of lupus nephritis. *Front Immunol*. 2023;14:1326836. doi:10.3389/fimmu.2023.1326836
68. Bruschi M, La Porta E, Panfoli I, et al. Proteomic profile of mesothelial exosomes isolated from peritoneal dialysis effluent of children with focal segmental glomerulosclerosis. *Sci Rep*. 2021;11(1):20807. doi:10.1038/s41598-021-00324-4
69. Obexer R, Nassir M, Moody ER, Baran PS, Lovelock SL. Modern approaches to therapeutic oligonucleotide manufacturing. *Science*. 2024;384(6692):eadl4015. doi:10.1126/science.adl4015
70. Takakusa H, Iwazaki N, Nishikawa M, Yoshida T, Obika S, Inoue T. Drug metabolism and pharmacokinetics of antisense oligonucleotide therapeutics: typical profiles, evaluation approaches, and points to consider compared with small molecule drugs. *Nucleic Acid Ther*. 2023;33(2):83–94. doi:10.1089/nat.2022.0054
71. Friedrich M, Aigner A. Therapeutic siRNA: state-of-the-art and future perspectives. *BioDrugs*. 2022;36(5):549–571. doi:10.1007/s40259-022-00549-3
72. Moazzam M, Zhang M, Hussain A, Yu X, Huang J, Huang Y. The landscape of nanoparticle-based siRNA delivery and therapeutic development. *Mol Ther*. 2024;32(2):284–312. doi:10.1016/j.ymthe.2024.01.005
73. Ramasamy T, Ruttala HB, Munusamy S, Chakraborty N, Kim JO. Nano drug delivery systems for antisense oligonucleotides (ASO) therapeutics. *J Control Release*. 2022;352:861–878. doi:10.1016/j.jconrel.2022.10.050
74. Hörberg J, Carlesso A, Reymer A. Mechanistic insights into ASO-RNA complexation: advancing antisense oligonucleotide design strategies. *Mol Ther Nucleic Acids*. 2024;35(4):10235. doi:10.1016/j.omtn.2024.102351
75. Kilikevicius A, Meister G, Corey DR. Reexamining assumptions about miRNA-guided gene silencing. *Nucleic Acids Res*. 2022;50(2):617–634. doi:10.1093/nar/gkab1256
76. Ho PTB, Clark IM, Le LTT. MicroRNA-based diagnosis and therapy. *Int J Mol Sci*. 2022;23(13):7167. doi:10.3390/ijms23137167
77. Gale DP, Gross O, Wang F, et al. A randomized controlled clinical trial testing effects of lademirsen on kidney function decline in adults with alport syndrome. *Clin J Am Soc Nephrol*. 2024;19(8):995–1004. doi:10.2215/CJN.0000000000000458
78. Bohrmann L, Burghardt T, Haynes C, Saatchi K, Häfeli UO. Aptamers used for molecular imaging and theranostics - recent developments. *Theranostics*. 2022;12(9):4010–4050. doi:10.7150/thno.72949
79. Ji D, Feng H, Liew SW, Kwok CK. Modified nucleic acid aptamers: development, characterization, and biological applications. *Trends Biotechnol*. 2023;41(11):1360–1384. doi:10.1016/j.tibtech.2023.05.005
80. Krylova SV, Feng D. The machinery of exosomes: biogenesis, release, and uptake. *Int J Mol Sci*. 2023;24(2):1337. doi:10.3390/ijms24021337
81. Mullen M, Williams K, LaRocca T, et al. Mechanical strain drives exosome production, function, and miRNA cargo in C2C12 muscle progenitor cells. *J Orthop Res*. 2023;41(6):1186–1197. doi:10.1002/jor.25467
82. Thielmann M, Corteville D, Szabo G, et al. Teprasiran, a Small Interfering RNA, for the Prevention of Acute Kidney Injury in High-Risk Patients Undergoing Cardiac Surgery: a Randomized Clinical Study. *Circulation*. 2021;144(14):1133–1144. doi:10.1161/CIRCULATIONAHA.120.053029
83. Jung HR, Lee J, Hong SP, et al. Targeting the mA RNA methyltransferase METTL3 attenuates the development of kidney fibrosis. *Exp Mol Med*. 2024;56(2):355–369. doi:10.1038/s12276-024-01159-5
84. Dai Y, Chen Y, Mo D, et al. Inhibition of ACSL4 ameliorates tubular ferroptotic cell death and protects against fibrotic kidney disease. *Commun Biol*. 2023;6(1):907. doi:10.1038/s42003-023-05272-5

85. Gerrits T, Dijkstra KL, Bruijn JA, Scharpfenecker M, Bijkerk R, Baelde HJ. Antisense oligonucleotide-mediated terminal intron retention of endoglin: a potential strategy to inhibit renal interstitial fibrosis. *Biochim Biophys Acta Mol Basis Dis*. 2024;1870(5):167186. doi:10.1016/j.bbadis.2024.167186
86. Phanish MK, Heidebrecht F, Jackson M, Rigo F, Dockrell MEC. Targeting alternative splicing of fibronectin in human renal proximal tubule epithelial cells with antisense oligonucleotides to reduce EDA+ fibronectin production and block an autocrine loop that drives renal fibrosis. *Exp Cell Res*. 2024;442(1):114186.
87. Kaneko S, Yanai K, Ishii H, et al. miR-122-5p regulates renal fibrosis in Vivo. *Int J Mol Sci*. 2022;23(23):15423. doi:10.3390/ijms232315423
88. Yoon YM, Go G, Yun CW, Lim JH, Lee JH, Lee SH. *Melatonin suppresses renal cortical fibrosis by inhibiting cytoskeleton reorganization and mitochondrial dysfunction through regulation of miR*-4516. *Int J Mol Sci*. 2020;21(15):5323. doi:10.3390/ijms21155323
89. Um JE, Park JT, Nam BY, et al. Periostin-binding DNA aptamer treatment attenuates renal fibrosis under diabetic conditions. *Sci Rep*. 2017;7(1):8490. doi:10.1038/s41598-017-09238-6
90. Zhu G, Pei L, Lin F, et al. Exosomes from human-bone-marrow-derived mesenchymal stem cells protect against renal ischemia/reperfusion injury via transferring miR-199a-3p. *J Cell Physiol*. 2019;234(12):23736–23749. doi:10.1002/jcp.28941
91. Grange C, Tritta S, Tapparo M, et al. Stem cell-derived extracellular vesicles inhibit and revert fibrosis progression in a mouse model of diabetic nephropathy. *Sci Rep*. 2019;9(1):4468. doi:10.1038/s41598-019-41100-9
92. Almeida A, Lira R, Oliveira M, et al. Bone marrow-derived mesenchymal stem cells transplantation ameliorates renal injury through anti-fibrotic and anti- inflammatory effects in chronic experimental renovascular disease. *Biomed J*. 2022;45(4):629–641. doi:10.1016/j.bj.2021.07.009
93. Hu XM, Shen N, Liu AQ, et al. Bone marrow mesenchymal stem cell-derived exosomal mir-34c-5p ameliorates rif by inhibiting the core fucosylation of multiple proteins. *Mol Ther*. 2022;30(2):763–781. doi:10.1016/j.ymthe.2021.10.012
94. Xu S, Cheuk YC, Jia Y, et al. Bone marrow mesenchymal stem cell-derived exosomal mir-21a-5p alleviates renal fibrosis by attenuating glycolysis by targeting PFKM. *Cell Death Dis*. 2022;13(10):876. doi:10.1038/s41419-022-05305-7
95. Wei W, Guo X, Gu L, et al. Bone marrow mesenchymal stem cell exosomes suppress phosphate-induced aortic calcification via SIRT6-HMGB1 deacetylation. *Stem Cell Res Ther*. 2021;12(1):235. doi:10.1186/s13287-021-02307-8
96. Liu W, Hu CH, Zhang BY, Li MX, Deng FX, Zhao SP. Exosomal microRNA-342-5p secreted from adipose-derived mesenchymal stem cells mitigates acute kidney injury in sepsis mice by inhibiting TLR9. *Biol Proced Online*. 2023;25(1):10. doi:10.1186/s12575-023-00198-y
97. Cao S, Huang Y, Dai ZH, et al. Circular RNA mmu_circ_0001295 from hypoxia pretreated adipose-derived mesenchymal stem cells (ADSCs) exosomes improves outcomes and inhibits sepsis-induced renal injury in a mouse model of sepsis. *Bioengineered*. 2022;13(3):6323–6331. doi:10.1080/21655979.2022.2044720
98. Shimamura Y, Furuhashi K, Tanaka A, et al. Mesenchymal stem cells exert renoprotection via extracellular vesicle-mediated modulation of M2 macrophages and spleen-kidney network. *Commun Biol*. 2022;5(1):753. doi:10.1038/s42003-022-03712-2
99. Ferguson CM, Farahani RA, Zhu XY, et al. Mesenchymal stem/stromal cell-derived extracellular vesicles elicit better preservation of the intra-renal microvasculature than renal revascularization in pigs with renovascular disease. *Cells*. 2021;10(4):763. doi:10.3390/cells10040763
100. Duan YR, Luo QY, Wang Y, et al. Adipose mesenchymal stem cell-derived extracellular vesicles containing microRNA- 26a-5p target TLR4 and protect against Diabetic Nephropathy. *J Biol Chem*. 2020;295(37):12868–12884. doi:10.1074/jbc.RA120.012522
101. Hao Y, Miao J, Liu WJ, Cai KQ, Huang XL, Peng L. *Mesenchymal stem cell-derived exosomes carry microrna*-125a to protect against diabetic nephropathy by targeting histone deacetylase 1 and downregulating endothelin-1. *Diabetes Metab Syndr Obes*. 2021;14:1405–1418. doi:10.2147/DMSO.S286191
102. Xu DJ, Yuan L, Che MY, et al. Molecular mechanism of gan-song yin inhibiting the proliferation of renal tubular epithelial cells by regulating Mir-21-5p in adipocyte exosomes. *J Ethnopharmacol*. 2024;321:117530. doi:10.1016/j.jep.2023.117530
103. Convento MB, de Oliveira AS, Boim MA, Borges FT. Umbilical cord mesenchymal stem cell-derived extracellular vesicles as natural nanocarriers in the treatment of nephrotoxic injury in Vitro. *Cells*. 2024;13(19):1658. doi:10.3390/cells13191658
104. Toghiani R, Azimian Zavareh V, Najafi H, et al. Hypoxia-preconditioned WJ-MSC spheroid-derived exosomes delivering mir-210 for renal cell restoration in hypoxia-reoxygenation injury. *Stem Cell Res Ther*. 2024;15(1):240. doi:10.1186/s13287-024-03845-7
105. Yu YH, Chen ML, Guo QT, et al. Human umbilical cord mesenchymal stem cell exosome-derived miR-874-3p targeting Ripk1/Pgam5 attenuates kidney tubular epithelial cell damage. *Cell Mol Biol Lett*. 2023;28(1):12. doi:10.1186/s11658-023-00425-0
106. Liao CM, Luo T, von der Ohe J, de Juan Mora B, Schmitt R, Hass R. Human MSC-derived exosomes reduce cellular senescence in renal epithelial cells. *Int J Mol Sci*. 2021;22(24):13562. doi:10.3390/ijms222413562
107. Du T, Ju GQ, Zhou J, et al. Microvesicles derived from human umbilical cord mesenchyme promote m2 macrophage polarization and ameliorate renal fibrosis following partial nephrectomy via hepatocyte growth factor. *Hum Cell*. 2021;34(4):1103–1113. doi:10.1007/s13577-021-00525-z
108. Wang Y. Protective effect of miRNA-containing extracellular vesicles derived from mesenchymal stromal cells of old rats on renal f unction in chronic kidney disease. *Stem Cell Res Ther*. 2020;11(1):274. doi:10.1186/s13287-020-01792-7
109. Shi L, Hu Y, Zeng H, et al. Mesenchymal stem cell-derived extracellular vesicles ameliorate renal interstitial fibrosis via the miR-13474/ADAM17 Axis. *Sci Rep*. 2024;14(1):17703. doi:10.1038/s41598-024-67339-5
110. Li X, Liao J, Su X, et al. Human urine-derived stem cells protect against renal ischemia/reperfusion injury in a rat model via exosomal miR-146a-5p which targets IRAK1. *Theranostics*. 2020;10(21):9561–9578. doi:10.7150/thno.42153
111. Grange C, Papadimitriou E, Dimuccio V, et al. Urinary extracellular vesicles carrying klotho improve the recovery of renal function in an acute tubular injury model. *Mol Ther*. 2024;32(11):4158–4159. doi:10.1016/j.ymthe.2024.09.018
112. Sun Z, Wu J, Bi Q, Wang W. Exosomal lncRNA TUG1 derived from human urine-derived stem cells attenuates renal ischemia/reperfusion injury by interacting with srsf1 to regulate ascl4-mediated ferroptosis. *Stem Cell Res Ther*. 2022;13(1):297. doi:10.1186/s13287-022-02986-x
113. Yang B, Wang J, Qiao J, et al. Circ DENND4C inhibits pyroptosis and alleviates ischemia-reperfusion acute kidney injury by exosomes secreted from human urine-derived stem cells. *Chem Biol Interact*. 2024;391:110922. doi:10.1016/j.cbi.2024.110922
114. Sun Y, Zhao Y, Lu Y, et al. Urinary stem cell-derived exocrine circrna atg7 regulates the socs1/stat3 signaling pathway through Mir-4500, Inhibits m1 macrophage polarization, and alleviates the progression of diabetes nephropathy. *Int Urol Nephrol*. 2024;56(4):1449–1463. doi:10.1007/s11255-023-03819-3

115. Lu W, Guo Y, Liu H, et al. The inhibition of fibrosis and inflammation in obstructive kidney injury via the miR-122-5p/SOX2 axis using USC-Exos. *Biomater Res*. 2024;28:0013. doi:10.34133/bmr.0013
116. Chi D, Chen Y, Xiang C, et al. Human amnion epithelial cells and their derived exosomes alleviate sepsis-associated acute kidney injury via mitigating endothelial dysfunction. *Front Med*. 2022;9:829606. doi:10.3389/fmed.2022.829606
117. Bruno S, Chiabotto G, Cedrino M, et al. Extracellular vesicles derived from human liver stem cells attenuate chronic kidney disease development in an in vivo experimental model of renal ischemia and reperfusion injury. *Int J Mol Sci*. 2022;23(3):1485. doi:10.3390/ijms23031485
118. Zhu Z, Han C, Xian S, et al. Placental mesenchymal stromal cells (PMSCs) and pmsc-derived extracellular vesicles (PMSC-EVs) attenuated renal fibrosis in rats with unilateral ureteral obstruction (UUO) by regulating CD4+ T cell polarization. *Stem Cells Int*. 2020;2020:2685820. doi:10.1155/2020/2685820
119. Khamis T, Alsemeh AE, Alanazi A, et al. Breast milk mesenchymal stem cells and/or derived exosomes mitigated adenine-induced nephropathy via modulating renal autophagy and fibrotic signaling pathways and their epigenetic regulations. *Pharmaceutics*. 2023;15(8):2149. doi:10.3390/pharmaceutics15082149
120. Liu L, Wang L, Wang H, et al. Niaodukang mixture inhibits micro-inflammation in ckd rats by enhancing mir-146a levels in enterogenous exosomes. *J Ethnopharmacol*. 2024;332:118318. doi:10.1016/j.jep.2024.118318
121. Večerić-Haler Ž, Erman A, Cerar A, et al. Improved protective effect of umbilical cord stem cell transplantation on cisplatin-induced kidney injury in mice pretreated with antithymocyte globulin. *Stem Cells Int*. 2016;2016:3585362. doi:10.1155/2016/3585362
122. Xie Q, Hao Y, Li N, et al. Cellular uptake of engineered extracellular vesicles: biomechanisms, engineered strategies, and disease treatment. *Adv Healthc Mater*. 2024;13(2):e2302280. doi:10.1002/adhm.202302280
123. Martínez-Santillán A, González-Valdez J. Novel technologies for exosome and exosome-like nanovesicle procurement and enhancement. *Biomedicines*. 2023;11(5):1487. doi:10.3390/biomedicines11051487
124. Mishra A, Singh P, Qayoom I, Prasad A, Kumar A. Current strategies in tailoring methods for engineered exosomes and future avenues in biomedical applications. *J Mater Chem B*. 2021;9(32):6281–6309. doi:10.1039/D1TB01088C
125. Sadeghi S, Tehrani FR, Tahmasebi S, Shafiee A, Hashemi SM. Exosome engineering in cell therapy and drug delivery. *Inflammopharmacology*. 2023;31(1):145–169. doi:10.1007/s10787-022-01115-7
126. Kim SH, Kim CH, Lee CH, et al. Glycoengineered stem cell-derived extracellular vesicles for targeted therapy of acute kidney injury. *Biomaterials*. 2025;318:123165. doi:10.1016/j.biomaterials.2025.123165
127. Zhang CY, Shang YN, Chen XN, et al. Supramolecular nanofibers containing arginine-glycine-aspartate (RGD) peptides boost therapeutic efficacy of extracellular vesicles in kidney repair. *ACS Nano*. 2020;14(9):12133–12147. doi:10.1021/acsnano.0c05681
128. Tang TT, Wang B, Li ZL, et al. Kim-1 targeted extracellular vesicles: a new therapeutic platform for RNAi to treat AKI. *J Am Soc Nephrol*. 2021;32(10):2467–2483. doi:10.1681/ASN.2020111561
129. Wang H, Wang B, Zhang A, et al. Exosome-mediated miR-29 transfer reduces muscle atrophy and kidney fibrosis in mice. *Mol Ther*. 2019;27(3):571–583. doi:10.1016/j.ymthe.2019.01.008
130. Gharehchelou B, Mehrarya M, Sefidbakht Y, et al. Mesenchymal stem cell-derived exosome and liposome hybrids as transfection nanocarriers of Cas9-GFP plasmid to HEK293T cells. *PLoS One*. 2025;20(1):e0315168. doi:10.1371/journal.pone.0315168
131. Wu P, Tang Y, Jin C, et al. Neutrophil membrane engineered hucmsc sevs alleviate cisplatin-induced aki by enhancing cellular uptake and targeting. *J Nanobiotechnology*. 2022;20(1):353. doi:10.1186/s12951-022-01574-8
132. Li SL, Wang YP, Wang ZJ, et al. Enhanced renoprotective effect of gdnf-modified adipose-derived mesenchymal stem cells on renal interstitial fibrosis. *Stem Cell Res Ther*. 2021;12(1):27. doi:10.1186/s13287-020-02049-z
133. Choi HY, Kim TY, Lee M, et al. Kidney mesenchymal stem cell-derived extracellular vesicles engineered to express erythropoietin improve renal anemia in mice with chronic kidney disease. *Stem Cell Rev Rep*. 2022;18(3):980–992. doi:10.1007/s12015-021-10141-x
134. Van Delen M, Derdelinckx J, Wouters K, Nelissen I, Cools N A systematic review and meta-analysis of clinical trials assessing safety and efficacy of human extracellular vesicle-based therapy. *J Extracell Vesicles*. 2024;13(7):e12458. doi:10.1002/jev2.12458
135. Wang L, Liang A, Huang J. Exendin-4-enriched exosomes from hucmscs alleviate diabetic nephropathy via gut microbiota and immune modulation. *Front Microbiol*. 2024;15:1399632. doi:10.3389/fmicb.2024.1399632
136. Zhang W, Yuan Y, Li X, et al. Orange-derived and dexamethasone-encapsulated extracellular vesicles reduced proteinuria and alleviated pathological lesions in iga nephropathy by targeting intestinal lymphocytes. *Front Immunol*. 2022;13:1047951. doi:10.3389/fimmu.2022.1047951
137. Lee SY, Park JM, Rhim WK, et al. Multifunctional extracellular vesicles and edaravone-loaded scaffolds for kidney tissue regeneration by activating GDNF/RET Pathway. *Nano Converg*. 2024;11(1):43–47. doi:10.1186/s40580-024-00450-5
138. Rai A, Claridge B, Lozano J, Greening DW. The discovery of extracellular vesicles and their emergence as a next-generation therapy. *Circ Res*. 2024;135(1):198–221. doi:10.1161/CIRCRESAHA.123.323054
139. Bahadorani M, Nasiri M, Dellinger K, Aravamudhan S, Zadegan R. Engineering exosomes for therapeutic applications: decoding biogenesis, content modification, and cargo loading strategies. *Int J Nanomed*. 2024;19: 7137–7164570–584. doi:10.2147/IJN.S464249
140. Seijas A, Cora D, Novo M, Al-Soufi W, Sánchez L, Arana ÁJ. CRISPR/Cas9 delivery systems to enhance gene editing efficiency. *Int J Mol Sci*. 2025;26(9):4420. doi:10.3390/ijms26094420
141. Allemailem KS, Almatroudi A, Rahmani AH, et al. Recent updates of the crispr/cas9 genome editing system: novel approaches to regulate its spatiotemporal control by genetic and physicochemical strategies. *Int J Nanomed*. 2024;19:5335–5363. doi:10.2147/IJN.S455574
142. Nassar W, El-Ansary M, Sabry D, et al. Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. *Biomater Res*. 2016;20:21. doi:10.1186/s40824-016-0068-0
143. Habibi A, Khosravi A, Soleimani M, Nejabat M, Dara M, Azarpira N. Efficacy of topical mesenchymal stem cell exosome in Sjögren's syndrome-related dry eye: a randomized clinical trial. *BMC Ophthalmol*. 2025;25(1):299. doi:10.1186/s12886-025-04078-9
144. Cavallari C, Dellepiane S, Fonsato V, et al. Online hemodiafiltration inhibits inflammation-related endothelial dysfunction and vascular calcification of uremic patients modulating miR-223 expression in plasma extracellular vesicles. *J Immunol*. 2019;202(8):2372–2383. doi:10.4049/jimmunol.1800747

145. Kwon HH, Yang SH, Lee J, et al. Combination treatment with human adipose tissue stem cell-derived exosomes and fractional CO2 laser for acne scars: a 12-week prospective, double-blind, randomized, split-face study. *Acta Derm Venereol*. 2020;100(18):adv00310. doi:10.2340/00015555-3666
146. Ren P, Qian F, Fu L, et al. Adipose-derived stem cell exosomes regulate Nrf2/Keap1 in diabetic nephropathy by targeting FAM129B. *Diabetol Metab Syndr*. 2023;15(1):149. doi:10.1186/s13098-023-01119-5
147. Xie X, Song Q, Dai C, et al. Clinical safety and efficacy of allogenic human adipose mesenchymal stromal cells-derived exosomes in patients with mild to moderate Alzheimer's disease: a phase I/II clinical trial. *Gen Psychiatr*. 2023;36(5):e101143. doi:10.1136/gpsych-2023-101143
148. Li M, Huang H, Wei X, et al. Clinical investigation on nebulized human umbilical cord MSC-derived extracellular vesicles for pulmonary fibrosis treatment. *Signal Transduct Target Ther*. 2025;10(1):179. doi:10.1038/s41392-025-02262-3
149. Jangam TC, Desai SA, Patel VP, Pagare NB, Raut ND. Exosomes as therapeutic and diagnostic tools: advances, challenges, and future directions. *Cell Biochem Biophys*. 2025. doi:10.1007/s12013-025-01730-5
150. Kim HI, Park J, Zhu Y, Wang X, Han Y, Zhang D. Recent advances in extracellular vesicles for therapeutic cargo delivery. *Exp Mol Med*. 2024;56(4):836–849. doi:10.1038/s12276-024-01201-6
151. Liangsupree T, Multia E, Riekkola ML. Modern isolation and separation techniques for extracellular vesicles. *J Chromatogr A*. 2021;1636:461773. doi:10.1016/j.chroma.2020.461773
152. Gao M, Cai JR, Zitkovsky HS, Chen B, Guo LF. Comparison of yield, purity, and functional properties of large-volume exosome isolation using ultrafiltration and polymer-based precipitation. *Plast Reconstr Surg*. 2022;149(3):638–649. doi:10.1097/PRS.0000000000008830
153. Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? *Int J Mol Sci*. 2020;21(18):6466. doi:10.3390/ijms21186466
154. Buschmann D, Mussack V, Byrd JB. Separation, characterization, and standardization of extracellular vesicles for drug delivery applications. *Adv Drug Deliv Rev*. 2021;174:348–368. doi:10.1016/j.addr.2021.04.027
155. Guzman NA, Guzman DE. A two-dimensional affinity capture and separation mini-platform for the isolation, enrichment, and quantification of biomarkers and its potential use for liquid biopsy. *Biomedicines*. 2020;8(8):255. doi:10.3390/biomedicines8080255
156. Omrani M, Beyrampour-Basmenj H, Jahanban-Esfahlan R, et al. Global trend in exosome isolation and application: an update concept in management of diseases. *Mol Cell Biochem*. 2024;479(3):679–691. doi:10.1007/s11010-023-04756-6
157. Miceli RT, Chen TY, Nose Y, et al. Extracellular vesicles, RNA sequencing, and bioinformatic analyses: challenges, solutions, and recommendations. *J Extracell Vesicles*. 2024;13(12):e70005. doi:10.1002/jev2.70005
158. Zhang Y, Liu Q, Zhang X, et al. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. *J Nanobiotechnology*. 2022;20(1):279. doi:10.1186/s12951-022-01472-z
159. Robinson BP, Bass NR, Bhakt P, Spiliotis ET. Septin-coated microtubules promote maturation of multivesicular bodies by inhibiting their motility. *J Cell Biol*. 2024;223(8):e202308049. doi:10.1083/jcb.202308049
160. Ivanusic D, Madela K, Bannert N, Denner J. The large extracellular loop of CD63 interacts with gp41 of HIV-1 and is essential for establishing the virological synapse. *Sci Rep*. 2021;11(1):10011. doi:10.1038/s41598-021-89523-7
161. Neviani V, van Deventer S, Wörner TP, et al. Site-specific functionality and tryptophan mimicry of lipidation in tetraspanin CD9. *FEBS J*. 2020;287(24):5323–5344. doi:10.1111/febs.15295
162. Bailly C, Thuru X. Targeting of tetraspanin CD81 with monoclonal antibodies and small molecules to combat cancers and viral diseases. *Cancers*. 2023;15(7):2186. doi:10.3390/cancers15072186
163. Mathieu M, Névo N, Jouve M, et al. Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking of CD63 and CD9. *Nat Commun*. 2021;12(1):4389. doi:10.1038/s41467-021-24384-2
164. Cheng W, Xu C, Su Y, et al. Engineered extracellular vesicles: a potential treatment for regeneration. *iScience*. 2023;26(11):108282. doi:10.1016/j.isci.2023.108282
165. Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: applications in regenerative medicine. *Cells*. 2021;10(8):1959. doi:10.3390/cells10081959
166. Upadhya D, Shetty AK. *MISEV*2023 provides an updated and key reference for researchers studying the basic biology and applications of extracellular vesicles. *Stem Cells Transl Med*. 2024;13(9):848–850. doi:10.1093/stcltm/szae052
167. Yuan F, Li YM, Wang Z. Preserving extracellular vesicles for biomedical applications: consideration of storage stability before and after isolation. *Drug Deliv*. 2021;28(1):1501–1509. doi:10.1080/10717544.2021.1951896
168. Bonsergent E, Grisard E, Buchrieser J, Schwartz O, Théry C, Lavieu G. Quantitative characterization of extracellular vesicle uptake and content delivery within mammalian cells. *Nat Commun*. 2021;12(1):1864–1867. doi:10.1038/s41467-021-22126-y
169. Sivanantham A, Jin Y. Impact of storage conditions on ev integrity/surface markers and cargos. *Life*. 2022;12(5):697–699. doi:10.3390/life12050697
170. Bosch S, de Beaurepaire L, Allard M, et al. trehalose prevents aggregation of exosomes and cryodamage. *Sci Rep*. 2016;6:36162. doi:10.1038/srep36162
171. Görgens A, Corso G, Hagey DW, et al. Identification of storage conditions stabilizing extracellular vesicles preparations. *J Extracell Vesicles*. 2022;11(6):e12238. doi:10.1002/jev2.12238
172. Arabpour M, Saghazadeh A, Rezaei N. Anti-inflammatory and M2 macrophage polarization-promoting effect of mesenchymal stem cell-derived exosomes. *Int Immunopharmacol*. 2021;97:107823. doi:10.1016/j.intimp.2021.107823
173. Zhu D, Liu S, Huang K, et al. Intrapericardial exosome therapy dampens cardiac injury via activating FOXO3. *Circ Res*. 2022;131(10):e135–e150. doi:10.1161/CIRCRESAHA.122.321384
174. Kim S, Lee SA, Yoon H, et al. Exosome-based delivery of super-repressor ικbα ameliorates kidney ischemia-reperfusion injury. *Kidney Int*. 2021;100(3):570–584. doi:10.1016/j.kint.2021.04.039
175. Hao B, Wang HD, Ren SH, et al. Exosomes derived from a mesenchymal-like endometrial regenerative cells ameliorate renal ischemia reperfusion injury through delivery of CD73. *Stem Cell Res Ther*. 2025;16(1):148. doi:10.1186/s13287-025-04275-9
176. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. *Cell Commun Signal*. 2022;20(1):145. doi:10.1186/s12964-022-00959-4