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# Urinary Exosomes: A Non-Invasive Window into Health and Disease

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***Abstract:***

The diagnosis and treatment of urinary exosomes, tiny vesicles expelled by cells in the urinary system, show enormous potential. Many of the compounds in them are very important and readily available without requiring surgical intervention. Apart from carrying proteins, lipids, and nucleic acids, these vesicles also carry nucleic acids displaying the physiological state of the urinary system and maybe other organs in the body. Here is another point of view on several diseases and disorders connected to health. Though standardizing isolation and characterizing techniques and having a thorough knowledge of the biological activities of these compounds continue to be difficult tasks, there is a great possibility for clinical applications. This work aims to provide a thorough investigation of the biology of urinary exosomes, covering their background, features, isolation techniques, and several uses in disease diagnosis and treatment. Apart from highlighting current advancements, it will also look at the possible future paths of this fast-changing field of work.

**Keywords:** Urinary exosomes, Personalized medicine, Non-invasive, Drug delivery.

## Introduction

Cells secrete small vesicles or sacs known as exosomes (Exs), which are absolutely essential for cell communication. Exs first came under notice in the 1960s, but their function remained unknown. In the 1980s, the term "exosome" was developed to characterize vesicles released by cells (1). Research over the past few decades has demonstrated their important roles in intercellular interaction and their great medicinal value. They emerge from the endosomal system of cells, where they branch out from a structure known as a multivesicular body (2). As the multivescular body unites with the cell membrane, this mechanism generates intraluminal vesicles, which are expelled as Exs. Reflecting the makeup of the cell from where they originated, these exosomes carry a broad spectrum of molecules, including proteins, lipids, and nucleic acids (3). Exs are messengers between cells, involved in a range of biological activities. They might move goods to recipient cells, so changing their behavior and purpose (4). Molecular movement could have a range of effects, including modulating immune responses, encouraging tissue healing, and even driving disease development. Because of their ability to transport specific molecules and change recipient cells, exosomes are under study for use in detection and treatments. They have, for instance, promise as biomarkers of medicine delivery vehicles and disease (5).



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Usually considered as a waste product, urine is a wealth of biological information. It includes exosomes, tiny vesicles made by cells that move various molecules, including proteins, lipids, and nucleic acids (6). From a range of cells in the urinary system including those lining the kidneys, bladder, and urethral opening urinary exosomes (UExs) and their cargo represent the physiological state of these cells; hence, they are a valuable source of information for treatment and research. Researching UExs is a rapidly growing field with enormous promise (7). UExs could provide a non-invasive means of assessing urinary tract health and function, including compounds unique to the cells from which they originated (8). For conditions like renal disease, where conventional diagnostic techniques might be invasive or insensitive, this is especially helpful. Researchers aim to find biomarkers for early diagnosis, track illness development, and potentially predict therapy response by studying the composition of UExs (9). The presentation will briefly explain the history and exploration of exosomes, review and evaluate the purification techniques and possible uses of UExs, present the most recent studies on UExs, and conclude with suggestions and conclusions for additional studies on UExs.

**History and development of UExs**

Various cells lining the urinary system that which comprises the kidneys, ureters, bladder, and urethra produce UExs. The kidneys' several cell types podocytes (specialist cells in the kidney's filtering units), proximal and distal tubule cells, and collecting duct cells 10 produce exosomes. The cells lining the bladder and urethra release exosomes as well into urine. Understanding these several sources is essential for diagnostic reasons since the cellular source of an Exe may affect its payload and possible function (11). Although their function was first unknown, the early 1980s marks the first discovery of exosomes in urine. Although these small vesicles were found in several body fluids, most notably urine, their significance remained unknown for a long period (12). Scientists first discovered exosomes as active participants in intercellular communication, capable of moving and distributing molecular signals between cells, later on. This realization piqued curiosity about the purposes of exosomes in health and disease, including those revealed in urine (13).

UEx generation is a well-organized process inside of cells. The process starts with the invagination of the cell membrane, which results in the formation of an early endosome (14). This initial endosome develops into a multivesicular body (MVB), a sphere-like structure that serves as the primary center for Exe synthesis. Smaller vesicles bud inward from the MVB's membrane to create intraluminal vesicles (ILVs). These ILVs enclose a range of components, including proteins, lipids, and nucleic acids, which will ultimately form the Exe cargo (14). The MVB's fusing with the cell membrane marks the last stage of exosome manufacture. This fusion exes ILVs into the extracellular area, today known as exosomes. Particularly under close control are the specific mechanisms driving this process, which include a complicated interaction of proteins and signaling pathways. The quantity and content of Exe generated may all be influenced by cellular stress, inflammation, and changes in the immediate surroundings. Understanding these regulatory systems is critical for unlocking exosomes' diagnostics and therapeutic potential (15).

**Properties of UExs**

UEXs are tiny vesicles with a diamiter between 30 and 150 nm, much as exosomes from many sources. This small scale determines their capacity to pass through bodily fluids and interact with cells (16). Research on electron microscopy and nanoparticle monitoring lets one find UEx size and concentration. It is crucial to underline that the individual's health state and the isolation technique applied determine the size distribution of urine exosomes (17). Their unique qualities and difference from other urinary elements come from this size range (18). Surfaces of UExs are covered in several compounds including carbohydrates, proteins, and fats. Crucially for biological activity, these outside molecules interact with target cells, affect absorption and cargo movement, and so enable Often used as EXs biomarkers, tetraspanins (CD9, CD63, and CD81) are prominent surface proteins found on urine exosomes. Adhesion molecules enable cell-cell contacts and other crucial surface proteins are signal transduction proteins, which could start signaling pathways in recipient cells. The cell of origin inside the urinary tract could influence the composition of surface proteins, so reflecting the physiological condition of the individual (19). Apart from enclosing their cargo, the lipid bilayer membrane of urinary exosomes maintains their stability and protects their contents from degradation. Including ceramide and cholesterol, UExs could have more lipids than the parent cell (20). These lipids could alter membrane fluidity and curvature, so influencing the exosome formation and operation. Moreover adding to the biological consequences of the exosome could be some lipids functioning as signaling molecules. The structural integrity of the exosome and supportive interactions with other biological components depend on the lipid content (21).

**Isolation and separation of UExs**

The different composition of urine makes

separating EXs from it a special challenge. Some elements found in urine, including proteins, salts, and extracellular trash, could hinder EX's purification (22). Hence, effective EX separation methods have to not only eliminate EXs from contaminants but also preserve their structural integrity and usefulness. There have been established several strategies for this goal, each with special advantages and drawbacks. Four groups could define these methods: microfluidic (23), precipitation-based, chromatography-based, and ultracentrifugation-based.

Still, a common method for isolating EXs is ultracentrifugation. A common starting point, differential centrifugation is a sequence of centrifugation operations carried out at progressively faster rates to remove larger particles, including cells and cellular waste (24). Ultracentrifugation often at rates more than 100,000 x g then follows to pellet the EXs. Although ultracentrifugation is simple and could generate many EXs, it may also remove other vesicles and protein clusters, so reducing the quality of purity (25). Furthermore, detrimental to EXs are strong centrifugal forces, so compromising their efficacy. Density gradient ultracentrifugation is a variation of this technique whereby EXs, depending on buoyant density, are further separated using a density gradient medium, so improving purity (26).

A less complex and more time-consuming choice than ultracentrifugation is precipitation methods. These methods work by adding a polymer, like polyethylene glycol (PEG), to a urine sample, which causes the EXs to settle out of the liquid. Then centrifugation can help to recover the precipitated EXs. Although precipitation techniques may be scaled up and are generally easy to use, they usually offer less purity than ultracentrifugation. PEG precipitation may cause other components from urine to co-precipitate, thus additional purification procedures are needed (28). But new developments in precipitation solutions and techniques have raised the quality and volume of EXs generated with this technique (28).

EXs benefit from more accuracy and purity provided by chromatography-based techniques. Size-exclusion chromatography (SEC) separates EXs by size using a column loaded with porous beads (29). Smaller than most other components of urine, EXs elute later from the column. SEC is a mild method for preserving EX's integrity and effectively eliminating 30 contaminated proteins. Other chromatography techniques, such as affinity chromatography and ion exchange, can also filter EXs depending on their outer charge or special binding capacity (31). Conversely, chromatography techniques could be more complex and call for specialized tools (31). Emerging microfluidic technologies offer possible fresh approaches for EX separation. These techniques produce quite accurate and efficient EXs separations by using microchannels and microstructures to control fluids and particles. Among the several separation methods used in microfluidic systems are dielectrophoresis, inertial focusing, and deterministic lateral displacement (32). These techniques are appealing for clinical use since they may be mechanized and have a great success rate. Microfluidic technologies have the potential to transform EXs separation from urine (33), even if their development is still under progress. The particular application as well as the necessary purity and yield define the method used to separate EXs. Ultracentrifugation with density gradient or chromatography-based techniques is used for applications including proteomic research or pharmaceutical development requiring great purity (34). Applications needing a high yield, such as diagnostic tests, could call for precipitation or ultracentrifugation. The most recent techniques, such as microfluidics, are perfect for therapeutic environments since they allow great throughput and automation (33). As research on this topic continues, EXs isolation techniques are expected to develop, so enabling more accurate and effective extraction of these vital biological entities from urine.

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formation and operation. Moreover, adding to the biological consequences of the exosome could be some lipids functioning as signaling molecules. The structural integrity of the exosome and supportive interactions with other biological components depend on the lipid content (21).

**Clinical application of UExs**

Especially in diagnosis and customized treatment, UExs show outstanding therapeutic value. Coming from many cells in the urinary tract and carrying molecular cargo reflecting the physiological condition of these cells, they provide a non-invasive window into the health and operation of the urinary system. For conditions like renal disease, where conventional diagnostic techniques could be intrusive or lacking in sensitivity, this is especially helpful (35). Analyzing the contents of UExs—which include proteins, lipids, and nucleic acids—clinicians hope to find biomarkers for early disease diagnosis, track disease development, predict therapy response, and even customize therapy regimens (36). UEx's research is a logical substitute for tissue biopsies or other invasive procedures since urine extraction is non-invasive.

One of the most likely clinically useful UExs is that for kidney disease diagnosis and treatment (37). Considered a major global health concern, chronic kidney disease (CKD) usually advances silently until major damage results. Even before traditional markers such as blood creatinine levels rise, unique protein and RNA biomarkers found in urinary exosomes have been shown to identify early kidney damage (37). For example, particular exosomal microRNAs have been identified as possible markers for diabetic nephropathy, a common diabetes condition sometimes causing kidney failure (38). Moreover, urine exosomes could offer specifics on the specific type of kidney disease, such as glomerular disease or tubular disease, thus allowing more exact diagnosis and customized treatment. Tracking changes in Exs composition over time might also help to assess therapy effectiveness and track disease development (39).

Apart from kidney diseases, urinary exosomes are under research as possible diagnostic tools for bladder and prostate cancer, among other urinary tract diseases. Different molecular fingerprints generated by cancer cells enable urine to be used in identification (35). These cancer-associated EXs could be lipids, nucleic acids, or tumor-specific proteins used as markers for first cancer diagnosis, disease staging, and response to treatment monitoring. For instance, researchers have identified specific exosomal proteins as potential markers for bladder cancer, suggesting a less invasive alternative to cystoscopy (40). Similarly, UExs are being investigated for their ability to track and identify prostate cancer, which may reduce the need for many biopsies. Both tissue and plasma exosomes significantly decreased PTEN pseudogene 1 (PTENP1) in bladder cancer. By competing with miR-17, this decrease may raise PTEN levels and slow down cancer growth. These findings highlight exosomal PTENP1 as a helpful marker for bladder cancer (BC) therapeutic identification and prognosis (36).

UExs could find use in clinical settings apart from diagnosis. Regarding therapy, they also seem promising. Researchers could produce Exs that carry drugs or other therapeutic agents, which would then target specific body cells (41). This customized treatment may increase therapy efficacy even while it helps to minimize side effects. We can target Exs containing anti-inflammatory drugs to specific kidney cells to treat inflammatory kidney diseases (42). Moreover, we could utilize the natural ability of exs to interact with other cells to induce urinary system tissue repair and regeneration. For instance, we could use Exs from healthy kidney cells to send regenerative signals to damaged kidney tissue (42). Conversion of research results into therapeutic uses depends on the development of uniform methods for Exs separation, characterization, and analysis. Massive clinical studies are needed to prove the clinical worth of UExs and to assess their detection and curative potential (43). Urinary exosomes have the potential to alter the detection and treatment of a wide range of diseases, particularly those affecting the urinary system, as technology develops and our knowledge of Exs biology improves. Along with the abundance of biological data kept inside exosomes, the non-invasive character of urine collecting makes them a very appealing instrument for customized treatment and improved patient care (37). Particularly in diagnostics and tailored treatment, UExs show outstanding therapeutic value. They offer a non-invasive window into the health and operation of the urinary system since they come from many cells in the urinary tract and carry molecular cargo reflecting the physiological condition of these cells. For conditions like renal disease, where conventional diagnostic techniques may be invasive or lacking in sensitivity, this information is particularly helpful (35).

By looking at what UExs contain—like proteins, lipids, and nucleic acids—doctors aim to find signs of disease that can help them diagnose early, monitor how the disease progresses, predict how well treatments will work, and even tailor treatment plans to individual patients. Urine extraction is non-invasive; thus, UEx research is a reasonable replacement for other invasive surgeries or tissue biopsies.

UExs have one of the most possible clinical applications—that of kidney disease identification

and treatment (37). A major worldwide health issue, chronic kidney disease (CKD) often advances silently until significant damage results. Unique protein and RNA biomarkers found in urinary exosomes have been shown to detect early kidney damage, even before conventional markers such blood creatinine levels rise (37). For instance, specific exosomal microRNAs have been found to be potential markers for diabetic nephropathy, a common diabetes disorder sometimes leading to kidney failure (38). Further, urine exosomes may offer information about the exact kind of kidney illness, such as glomerular disease or tubular disease, allowing for more precise diagnosis and individualized therapy. Tracking changes in Exs composition over time could also help to assess therapy efficacy and track disease development (39).

Apart from kidney disease, urinary exosomes are under investigation as potential diagnostic tools for other urinary tract diseases, including bladder and prostate cancer. Cancer cells produce different molecular fingerprints that enable urine identification (35). These cancer-associated Exs could be lipids, nucleic acids, or tumor-specific proteins used as markers for first cancer diagnosis, disease staging, and response to therapy monitoring. For instance, researchers have identified some exosomal proteins as potential markers for bladder cancer, suggesting a less invasive alternative to cystoscopy (40). Likewise, researchers are studying UExs for their ability to detect and monitor prostate cancer, which may reduce the need for multiple biopsies. Both tissue and plasma exosomes significantly reduced PTEN pseudogene 1 (PTENP1) in bladder cancer. Acting as a competitive endogenous RNA (ceRNA) for miR-17, this drop may raise PTEN levels by stopping cancer development. These findings suggest that exosomal PTENP1 could be a useful marker for identifying and predicting treatment outcomes in bladder cancer. UExs could find clinical application outside of diagnosis. They also show promise in terms of therapy. We could engineer exosomes to transport drugs or other therapeutic agents, which would then target specific body cells (41). While reducing negative effects, this tailored administration may improve therapy efficacy. We could target specific kidney cells with Exs containing anti-inflammatory drugs to treat inflammatory kidney diseases (42). Moreover, we could utilize the natural ability of exs to interact with other cells to induce urinary system tissue repair and regeneration. For instance, we could use Exs from healthy kidney cells to send regenerative signals to damaged kidney tissue (42). Conversion of research results into therapeutic uses depends on the development of uniform methods for Exs separation, characterization, and analysis. Massive clinical studies are needed to prove the clinical worth of UExs and to assess their detection and curative potential (43). Urinary exosomes have the potential to alter the detection and treatment of a wide range of diseases, particularly those affecting the urinary system, as technology develops and our knowledge of Exs biology improves. Along with the abundance of biological data kept inside exosomes, the non-invasive nature of urine collection makes them a quite appealing tool for customized treatment and improved patient care (37).

**UExs and bladder cancer**

Rising above more invasive treatments past, UExs are now recognized as a potential non-invasive BC detection and treatment tool. BC cells secrete Exs into the urine, much as many other types of cells do. These tiny vesicles carry molecules reflecting the characteristics of the tumor, including proteins, nucleic acids (including microRNAs), lipids, and metabolites (44). Acting as the fingerprint of the tumor, this cargo offers vital information on its existence, stage, aggressiveness, and most likely reaction to treatment. The ease of collecting urine specimens makes UExs screening particularly interesting for BC; it enables repeated evaluations over time free from the pain and hazards related with cystoscopy or tissue biopsy (45). Great promise exists for this non-invasive approach to increase fast detection rates, reduce the need for invasive procedures, and customize treatment plans for BC patients (46). Many studies have revealed especially exosomal markers in urine linked to BC. Usually overproduced in BC cells, proteins including cytokeratin 20 (CK20) and uroplakin 1A (UPK1A), a main component of the urothelium (the bladder lining), have been reported to be abundant in UExs from patients with BC (47). Usually helping the bladder lining to develop and function, these proteins are lost into the urine via exosomes when cancer strikes. Likewise, UExs have exposed some microRNAs—small non-coding RNAs that affect gene expression—as possible biomarkers (48). Some microRNAs, including miR-21 and miR-141, are routinely raised in BC and their presence in UExs could point to both the existence and stage of the disease. These exosomal markers can be found with a sensitive and exact approach using ELISA (enzyme-linked immunosorbent assay), Western blotting, and PCR (polymerase chain reaction), so guiding bladder cancer detection and aggressiveness determination. Especially for high-risk people like smokers or those handling specific chemicals, the identification of these markers in urine samples provides the route for the development of non-invasive BC monitoring systems. Imagine a time when a simple urine test could alert a patient of early bladder cancer detection, so facilitating earlier intervention and improved outcomes (50).

Beyond obvious tracking of therapy response and

BC prognosis prediction, UExs hold promise. Exs' molecular payload could highlight specifics on the tumor's stage, grade, and metastatic potential. For example, exes from severe bladder cancer could have different sets of proteins and microRNAs than those from less aggressive tumors (51). These exosomal biomarkers allow doctors to estimate the likelihood of disease development and guide the development of a suitable course of treatment. Should a patient's UExs show a high concentration of markers linked to aggressive cancer, their doctor may advise a more extreme course of treatment from the start (52). Moreover, the research of UExs could assist in assessing the success of treatments comprising chemotherapy and immunotherapy. Variations in the form or concentration of exosomal markers across treatment could offer crucial information about the effectiveness of the drug and whether changes are required. If a patient is undergoing chemotherapy and their UExs still show high levels of cancer-associated microRNAs, this indicates that the treatment is ineffective and should be modified (53).

Furthermore, under research are UExs based on their detection and prognostic value as possible BC treatment targets and drug delivery systems. Therapeutic molecules, including proteins, siRNAs (small interfering RNAs), or drugs, could be produced and then transported to BC cells. Although this customized delivery method reduces negative effects on normal tissues, it may increase cancer therapy efficacy (54).

Exs, for example, could be loaded with chemotherapeutic drugs and targeted onto BC cells, so delivering the treatments exactly to the tumor and avoiding other areas of the body (55). Also, learning how Exs play a role in communication between cells in the tumor area can lead to new treatment ideas that aim to disrupt the signals that help tumors grow and spread. Researchers are looking at how bladder cancerous cells interact with surrounding cells and with one another, as well as how this interaction might be inhibited to prevent the cancer from spreading. Although their therapeutic uses are yet under research, urinary Exs have great future potential to improve BC treatment (57).

**Urinary exosomes and vaccine development**

Especially in the fields of infectious diseases and cancer, exosomes released by cells and spanning a range of biological molecules are under research as a possible vaccination platform. Their related biological compatibility, ability to cause immune system reactions, and delivery of particular antigens make them therefore interesting candidates (58). Part of a multi-phase development process for Exs-based vaccines, Exs are isolated and purified, then loaded with the appropriate antigens and injected to induce an immune response (59). Tumor cells, infections, or other targets connected to diseases could all be the antigen source. Exs protect these antigens from annihilation and help them to be delivered to antigen-presenting cells, so promoting a good immune response. This approach may generate more customized and safer vaccinations than the ones now used (59).

The ability of UExs to produce humoral and cellular immune responses is among the most important advantages of their use in vaccination studies. Humoral immunity refers to the generation of antibodies that can either mark cancer cells for destruction or neutralize infections. T lymphocytes are part of cellular immunity; they are triggered and can directly destroy cancerous or diseased cells (60). Exs can boost both immune system arms, so producing a more complete and long-lasting immune response. Studies have indicated that exosomes generated from tumor cells and loaded with tumor-associated antigens—for example, help cytotoxic T lymphocytes (CTLs), which can destroy cancer cells, to flourish. Preclinical cancer models have shown the promise of this strategy, implying that Exs-based vaccines might be helpful in the treatment of many cancer types (61).

UExs are suitable for many vaccine targets since their adaptability allows one to design them to carry a broad spectrum of antigens. Exosomes can load tumor-specific antigens—such as cancer-associated carbohydrates or mutant proteins—to set off an immune reaction against cancer (62). To boost protective immunity in infectious diseases, examples could include pathogen-derived antigens, including viral proteins or bacterial toxins. Moreover, exosomes can be altered to increase their immunogenicity by including adjuvant drugs meant to activate the immune system. Because of their adaptability, UExs provide a great forum for creating vaccines against many different diseases (63).

Preclinical research has seen many studies looking at the possibilities of exosome-based vaccines. Studies have demonstrated, for instance, that exosomes derived from melanoma cells loaded with melanoma-associated antigens can generate a strong anti-tumor immune response in mice, so causing tumor regression. In another study, mice given the vaccination (58) showed humoral and cellular immune responses when exposed to Exs, including HIV-1 antigens. TEXs isolated from IL-12-anchored transformed kidney cancer cells showed stronger anticancer properties in vitro (64) when compared to TEXs and IL-12 alone. Though mostly preclinical research, they offer compelling proof that UExs could help create successful vaccines. Clinical research is essential to assess in humans the safety and efficacy of Exs-based vaccinations. Ongoing studies indicate that UExs have great potential to transform vaccination development and improve human health (65).

**Advantages and challenges**

In therapy and detection, UExs have several interesting advantages. Gained only by urine collection, their non-invasive character presents an attractive substitute for more intrusive procedures, including blood samples or biopsies. Regular sampling made possible by this simplicity of access helps to monitor disease development or therapy response longitudinally (59). UExs also carry a variety of biological molecules, including proteins, lipids, and nucleic acids, which mirror the urinary tract's and maybe other areas of the body's physiological state (66). Exs in urine also help to be useful since their long-term stability prevents their cargo from degrading; hence, they are fit for analysis and storage. This molecular complexity provides a wealth of information ready for use in particular treatments, tailored medicine, and first disease diagnosis (67).

There are plenty of fast-expanding possibilities for UEXs. Several diseases, such as those affecting the kidneys, bladder, and prostate, could benefit from their use in diagnostics for early identification and tracking (68). UExs could be used, for example, to identify markers for the degree of kidney disease development, BC recurrence, or prostate cancer severity, so enabling quick treatments and better outcomes for patients. Exs could be made to carry drugs or other medicinal compounds straight to target cells in treatments, thereby offering a more accurate and less damaging approach to therapy (69). Furthermore, Exs' natural ability for intercellular communication can encourage tissue renewal and regeneration. The possible uses of UExs go beyond the urinary tract since they can indicate systemic conditions and be used to diagnose and monitor different diseases, including neurological or cardiovascular ones (70).

UEXs have drawbacks even if they offer advantages. Standardizing Exs separation and characterization techniques is one of the main challenges. Variations in pre-analytical and analytical techniques could affect the purity, yields, and repeatability of Exs preparations, so complicating data comparison from many research studies (71). Ensuring data dependability and comparability thus depends on the development and validation of standardized methods for Exs separation, characterization, and analysis. Lack of knowledge on the biological roles of UExs adds still another challenge. Although UExs contain several compounds, their exact purposes in health and disease are unknown. We need further research to understand how UExs interact with destination cells and how their payload influences cellular functions (72).

The rather low concentration of Exs in urine especially in healthy individuals is another drawback. This could make it challenging to get enough Exs for specific purposes, such as medicinal distribution. Overcoming this limit calls for technological developments in Exs separation and enrichment processes (73). Furthermore, the diversity of UExs produced by several cell types in the urinary tract complicates data interpretation. First, we have to grasp the cellular origin of individual Exs if we are to fully appreciate their biological relevance. Ultimately, translating studies on urinary exosomes into clinical use calls for thorough validation in large-scale clinical trials, which are vital for proving the clinical relevance of Exs-based diagnostics and treatments as well as for establishing their safety and efficacy.

**Conclusion**

Urinary exosomes (UExs) offer a remarkable cutting edge in non-invasive diagnostics and personalized medicine by providing a wealth of information on the physiological and pathological states of the urinary system and possibly other organs. These tiny vesicles, filled with proteins, fats, and genetic material, are especially helpful for spotting diseases early, predicting outcomes, and tracking treatment in kidney disease, bladder cancer, and other urinary issues. They also reflect the state of the cells from which they originate. Despite significant advancements in our understanding of their biology and the development of numerous isolation techniques, standardization, purification, and clinical validation remain challenging. However, as innovative technologies like microfluidics advance and our understanding of exosomal biology expands, the incorporation of UExs into traditional clinical practice appears to be becoming more and more possible. Future research focused on improving isolation methods, raising biomarker specificity, and clarifying the functional roles of UExs will help us completely realize their diagnostic and therapeutic potential in precision medicine.

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**Ethics approval and consent to participate**

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**Conflict of Interest**

No conflict of interest was declared.

**Consent for publication**

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**References**

1.Lai JJ, Chau ZL, Chen SY, Hill JJ, Korpany KV, Liang NW, et al. Exosome processing and characterization approaches for research and technology development. Advanced Science. 2022;9(15):2103222.

2.Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R,

et al. Review on strategies and technologies for exosome isolation and purification. Frontiers in bioengineering and biotechnology. 2022;9:811971.
3.Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. Theranostics. 2017;7(3):789.
4.Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cellular and Molecular Life Sciences. 2018;75:193-208.
5.Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, et al. Progress, opportunity, and perspective on exosome isolation-efforts for efficient exosome-based theranostics. Theranostics. 2020;10(8):3684.
6.Decramer S, de Peredo AG, Breuil B, Mischak H, Monsarrat B, Bascands J-L, et al. Urine in clinical proteomics. Molecular & cellular proteomics. 2008;7(10):1850-62.
7.Street J, Koritzinsky E, Glispie D, Star R, Yuen P. Urine exosomes: an emerging trove of biomarkers. Advances in clinical chemistry. 2017;78:103-22.
8.Wang Y, Zhang M. Urinary exosomes: A promising biomarker for disease diagnosis. Laboratory Medicine. 2023;54(2):115-25.
9.Lee N, Canagasingham A, Bajaj M, Shanmugasundaram R, Hutton A, Bucci J, et al. Urine exosomes as biomarkers in bladder cancer diagnosis and prognosis: from functional roles to clinical significance. Frontiers in Oncology. 2022;12:1019391.
10.El Fekih R, Hurley J, Tadigotla V, Alghamdi A, Srivastava A, Coticchia C, et al. Discovery and validation of a urinary exosome mRNA signature for the diagnosis of human kidney transplant rejection. Journal of the American Society of Nephrology. 2021;32(4):994-1004.
11.Street JM, Koritzinsky EH, Glispie DM, Yuen PS. Urine exosome isolation and characterization. Drug Safety Evaluation: Methods and Protocols. 2017:413-23.
12.Dimov I, Jankovic Velickovic L, Stefanovic V. Urinary exosomes. The Scientific World Journal. 2009;9(1):1107-18.
13.Mizutani K, Kawakami K, Horie K, Fujita Y, Kameyama K, Kato T, et al. Urinary exosome as a potential biomarker for urinary tract infection. Cellular microbiology. 2019;21(7):e13020.
14.Gonzales PA, Zhou H, Pisitkun T, Wang NS, Star RA, Knepper MA, et al. Isolation and purification of exosomes in urine. The Urinary Proteome: Methods and Protocols. 2010:89-99.
15.Cao Y, Shi Y, Yang Y, Wu Z, Peng N, Xiao J, et al. Urinary exosomes derived circRNAs as biomarkers for chronic renal fibrosis. Annals of Medicine. 2022;54(1):1966-76.
16.Blijdorp CJ, Tutakhel OA, Hartjes TA, van den Bosch TP, van Heugten MH, Rigalli JP, et al. Comparing approaches to normalize, quantify, and characterize urinary extracellular vesicles. Journal of the American Society of Nephrology. 2021;32(5):1210-26.
17.Xiang X, Guan F, Jiao F, Li H, Zhang W, Zhang Y, et al. A new urinary exosome enrichment method by a combination of ultrafiltration and TiO 2 nanoparticles. Analytical Methods. 2021;13(13):1591-600.
18.Lee H, Kang SJ, Lee J, Park KH, Rhee WJ. Isolation and characterization of urinary extracellular vesicles from healthy donors and patients with castration-resistant prostate cancer. International Journal of Molecular Sciences. 2022;23(13):7134.
19.Deng F, Miller J. A review on protein markers of exosome from different bio-resources and the antibodies used for characterization. Journal of Histotechnology. 2019;42(4):226-39.
20.Gheinani AH, Vögeli M, Baumgartner U, Vassella E, Draeger A, Burkhard FC, et al. Improved isolation strategies to increase the yield and purity of human urinary exosomes for biomarker discovery. Scientific reports. 2018;8(1):3945.
21.Asvapromtada S, Sonoda H, Kinouchi M, Oshikawa S, Takahashi S, Hoshino Y, et al. Characterization of urinary exosomal release of aquaporin-1 and-2 after renal ischemia-reperfusion in rats. American Journal of Physiology-Renal Physiology. 2018;314(4):F584-F601.
22.Zhang M, Jin K, Gao L, Zhang Z, Li F, Zhou F, et al. Methods and technologies for exosome isolation and characterization. Small Methods. 2018;2(9):1800021.
23.Ludwig N, Whiteside TL, Reichert TE. Challenges in exosome isolation and analysis in health and disease. International journal of molecular sciences. 2019;20(19):4684.
24.Yakubovich E, Polischouk A, Evtushenko V. Principles and problems of exosome isolation from biological fluids. Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology. 2022;16(2):115-26.
25.Liu W-z, Ma Z-j, Kang X-w. Current status and outlook of advances in exosome isolation. Analytical and Bioanalytical Chemistry. 2022;414(24):7123-41.
26.Soares Martins T, Catita J, Martins Rosa I, AB da Cruz e Silva O, Henriques AG. Exosome isolation from distinct biofluids using precipitation and column-based approaches. PloS one. 2018;13(6):e0198820.
27.Coughlan C, Bruce KD, Burgy O, Boyd TD, Michel CR, Garcia-Perez JE, et al. Exosome isolation by ultracentrifugation and precipitation and techniques for downstream analyses. Current protocols in cell biology. 2020;88(1):e110.
28.Xu K, Jin Y, Li Y, Huang Y, Zhao R. Recent progress of exosome isolation and peptide recognition-

guided strategies for exosome research. Frontiers in Chemistry. 2022;10:844124.
29.Sidhom K, Obi PO, Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? International journal of molecular sciences. 2020;21(18):6466.
30.Stranska R, Gysbrechts L, Wouters J, Vermeersch P, Bloch K, Dierickx D, et al. Comparison of membrane affinity-based method with size-exclusion chromatography for isolation of exosome-like vesicles from human plasma. Journal of translational medicine. 2018;16:1-9.
31.Navajas R, Corrales FJ, Paradela A. Serum exosome isolation by size-exclusion chromatography for the discovery and validation of preeclampsia-associated biomarkers. Proteomics for Biomarker Discovery: Methods and Protocols. 2019:39-50.
32.Le M-CN, Fan ZH. Exosome isolation using nanostructures and microfluidic devices. Biomedical Materials. 2021;16(2):022005.
33.Wang J, Ma P, Kim DH, Liu B-F, Demirci U. Towards microfluidic-based exosome isolation and detection for tumor therapy. Nano Today. 2021;37:101066.
34.Shen J, Ma Z, Xu J, Xue T, Lv X, Zhu G, et al. Exosome isolation and detection: from microfluidic chips to nanoplasmonic biosensor. ACS Applied Materials & Interfaces. 2024;16(18):22776-93.
35.Liu J, Zhijin Z, Zhang W, Niraj M, Yang F, Changcheng G, et al. Urinary exosomes: Potential diagnostic markers and application in bladder cancer. Heliyon. 2024;10(12).
36.Song Q, Yu H, Han J, Lv J, Lv Q, Yang H. Exosomes in urological diseases-Biological functions and clinical applications. Cancer Letters. 2022;544:215809.
37.Gang D, Yu CJ, Zhu S, Zhu P, Nasser M. Application of mesenchymal stem cell-derived exosomes in kidney diseases. Cellular Immunology. 2021;364:104358.
38.Qin Z, Hu H, Sun W, Chen L, Jin S, Xu Q, et al. miR-224-5p contained in urinary extracellular vesicles regulates PD-L1 expression by inhibiting cyclin D1 in renal cell carcinoma cells. Cancers. 2021;13(4):618.
39.Sharma S, Rana R, Prakash P, Ganguly NK. Drug target therapy and emerging clinical relevance of exosomes in meningeal tumors. Molecular and Cellular Biochemistry. 2024;479(1):127-70.
40. Wen J, Yang T, Mallouk N, Zhang Y, Li H, Lambert C, et al. Urinary exosomal CA9 mRNA as a novel liquid biopsy for molecular diagnosis of bladder cancer. International Journal of Nanomedicine. 2021:4805-11.
41.Rajput A, Varshney A, Bajaj R, Pokharkar V. Exosomes as new generation vehicles for drug delivery: biomedical applications and future perspectives. Molecules. 2022;27(21):7289.
42.Guo ZY, Tang Y, Cheng YC. Exosomes as targeted delivery drug system: advances in exosome loading, surface functionalization and potential for clinical application. Current Drug Delivery. 2024;21(4):473-87.
43.Vitorino R, Ferreira R, Guedes S, Amado F, Thongboonkerd V. What can urinary exosomes tell us? Cellular and Molecular Life Sciences. 2021;78:3265-83.
44.Hiltbrunner S, Mints M, Eldh M, Rosenblatt R, Holmström B, Alamdari F, et al. Urinary exosomes from bladder cancer patients show a residual cancer phenotype despite complete pathological downstaging. Scientific reports. 2020;10(1):5960.
45.Beckham CJ, Olsen J, Yin P-N, Wu C-H, Ting H-J, Hagen FK, et al. Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression. The Journal of urology. 2014;192(2):583-92.
46.Elsharkawi F, Elsabah M, Shabayek M, Khaled H. Urine and serum exosomes as novel biomarkers in detection of bladder cancer. Asian Pacific journal of cancer prevention: APJCP. 2019;20(7):2219.
47.Yazarlou F, Modarressi MH, Mowla SJ, Oskooei VK, Motevaseli E, Tooli LF, et al. Urinary exosomal expression of long non-coding RNAs as diagnostic marker in bladder cancer. Cancer management and research. 2018:6357-65.
48.Wei X, Cai L, Chen H, Shang L, Zhao Y, Sun W. Noninvasive multiplexed analysis of bladder cancer-derived urine exosomes via janus magnetic microspheres. Analytical Chemistry. 2022;94(51):18034-41.
49.Øverbye A, Skotland T, Koehler CJ, Thiede B, Seierstad T, Berge V, et al. Identification of prostate cancer biomarkers in urinary exosomes. Oncotarget. 2015;6(30):30357.
50.Long C, Shi H, Li J, Chen L, Lv M, Tai W, et al. The diagnostic accuracy of urine-derived exosomes for bladder cancer: a systematic review and meta-analysis. World Journal of Surgical Oncology. 2024;22(1):285.
51.Wang Y-T, Shi T, Srivastava S, Kagan J, Liu T, Rodland KD. Proteomic analysis of exosomes for discovery of protein biomarkers for prostate and bladder cancer. Cancers. 2020;12(9):2335.
52.Geng H, Zhou Q, Guo W, Lu L, Bi L, Wang Y, et al. Exosomes in bladder cancer: novel biomarkers and targets. Journal of Zhejiang University-SCIENCE B. 2021;22(5):341-7.
53.Wang X, Song D, Zhu B, Jin Y, Cai C, Wang Z. Urinary exosomal mRNA as a biomarker for the diagnosis of bladder cancer. Anti-Cancer Drugs. 2024;35(4):362-70.
54.Zhu Q, Cheng L, Deng C, Huang L, Li J, Wang Y, et al. The genetic source tracking of human urinary exosomes. Proceedings of the National Academy

of Sciences. 2021;118(43):e2108876118.
55.Franzen CA, Simms PE, Van Huis AF, Foreman KE, Kuo PC, Gupta GN. Characterization of uptake and internalization of exosomes by bladder cancer cells. BioMed research international. 2014;2014(1):619829.
56.Leiblich A. Recent developments in the search for urinary biomarkers in bladder cancer. Current urology reports. 2017;18:1-6.
57.Panfoli I. Cancer exosomes in urine: a promising biomarker source. Translational cancer research. 2017;6(Suppl 8).
58.Huda MN, Nurunnabi M. Potential application of exosomes in vaccine development and delivery. Pharmaceutical Research. 2022;39(11):2635-71.
59.Tan D, Li G, Fu W, Lei C. Exosomes: The next frontier in vaccine development and delivery. Frontiers in Immunology. 2024;15:1435426.
60.El Safadi D, Mokhtari A, Krejbich M, Lagrave A, Hirigoyen U, Lebeau G, et al. Exosome-mediated antigen delivery: unveiling novel strategies in viral infection control and vaccine design. Vaccines. 2024;12(3):280.
61.Pordanjani PM, Bolhassani A, Milani A, Pouriayevali MH. Extracellular vesicles in vaccine development and therapeutic approaches for viral diseases. Process Biochemistry. 2023;128:167-80.
62.Behzadi P, Kim C-H, Pawlak EA, Algammal A. The innate and adaptive immune system in human urinary system. Frontiers Media SA; 2023. p. 1294869.
63.Seo J-W, Lee YH, Tae DH, Kim YG, Moon J-Y, Jung SW, et al. Development and validation of urinary exosomal microRNA biomarkers for the diagnosis of acute rejection in kidney transplant recipients. Frontiers in immunology. 2023;14:1190576.
64.Santos P, Almeida F. Exosome-based vaccines: history, current state, and clinical trials. Frontiers in Immunology. 2021;12:711565.
65.Dhar R, Bhattacharya B, Mandal D, Devi A, Thorat ND. Exosome-based cancer vaccine: A cutting-edge approach–Correspondence. International journal of surgery. 2022;108:106993.
66.Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Communication and Signaling. 2022;20(1):145.
67.Yang M, Wu SY. The advances and challenges in utilizing exosomes for delivering cancer therapeutics. Frontiers in pharmacology. 2018;9:735.
68.Yamashita T, Takahashi Y, Takakura Y. Possibility of exosome-based therapeutics and challenges in production of exosomes eligible for therapeutic application. Biological and Pharmaceutical Bulletin. 2018;41(6):835-42.
69.Li X, Corbett AL, Taatizadeh E, Tasnim N, Little JP, Garnis C, et al. Challenges and opportunities in exosome research—Perspectives from biology, engineering, and cancer therapy. APL bioengineering. 2019;3(1).
70.Yu W, Hurley J, Roberts D, Chakrabortty S, Enderle D, Noerholm M, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. Annals of Oncology. 2021;32(4):466-77.
71.Syn NL, Wang L, Chow EK-H, Lim CT, Goh B-C. Exosomes in cancer nanomedicine and immunotherapy: prospects and challenges. Trends in biotechnology. 2017;35(7):665-76.
72.Rezabakhsh A, Sokullu E, Rahbarghazi R. Applications, challenges and prospects of mesenchymal stem cell exosomes in regenerative medicine. Stem Cell Research & Therapy. 2021;12:1-8.
73.Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharmaceutica Sinica B. 2016;6(4):287-96.