# Extracellular Vesicles as Novel Players in Kidney Disease

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"Urine can provide us day by day, month by month, and year by year with a serial story of the major events within the kidney." Dr. Thomas Addis, pioneer in renal physiology, came to this conclusion in 1948. The discovery of urinary extracellular vesicles (uEVs) as molecular mimics of kidney cells has taken his view to a new level (Figure 1). uEVs include nano-sized vesicles that bud outward from the cell plasma membrane of healthy or dying cells (microvesicles or apoptotic bodies, respectively) or are excreted via multivesicular bodies in a regulated process (exosomes). True to Dr. Addis' view, uEVs contain disease- and site-specific markers from cells lining the kidney's tubules and urinary tract,[1] making them an invaluable addition to markers of kidney dysfunction such as serum creatinine and proteinuria, which are nonspecific and late to manifest. Evolving evidence demonstrates uEVs' potential to predict disease earlier than conventional markers. Indeed, the first uEV-based biomarker was recently Food and Drug Administration approved in the field of urology, where a uEV RNA signature serves as a noninvasive screening method for prostate cancer. Other than their role in diagnosis and prognosis, uEVs are increasingly studied as novel messengers in renal disease mechanisms and in the context of regenerative medicine. Recently, a position statement was published by the Urine Task Force of the International Society of Extracellular Vesicles to advance rigor and standardization of uEV analysis and accelerate clinical application of uEVs.[1]

## uEVs FOR DIAGNOSIS AND PROGNOSIS

The use of uEVs for diagnosis and prognosis in liquid biopsies hinges on the fact that extracellular vesicles (EVs) retain properties of the cell from which they are formed. uEV diagnostic studies can be broadly divided into two approaches: (*1*) approaches focused on single-EV analysis (for example, by Nanoparticle Tracking Analysis or flow cytometry) and (*2*) approaches focused on bulk EV analysis, such as proteomics or RNA profiling. The focus of single-EV approaches is generally enumeration of uEVs and targeted phenotyping, such as detecting EV's cell of origin and specific cargo from glomerular and tubular cells. Early studies suggested that elevations in uEV levels may be indicative of underlying disease. For example, urinary podocyte EVs were increased in diabetic kidney disease in advance of albuminuria,[2] and elevations in uEV subpopulations were reported in preeclampsia.[3] Many early studies are notable for heterogeneity in methodology and subsequent challenges with external validation. Several use suboptimal instrumentation, lack appropriate controls or antibody validation, and fail to include orthogonal approaches to confirm vesicle isolation. A recent study showcases a modern approach for uEV assessment by flow cytometry, which will be essential for the transition from research tool to clinical test.[4] A comprehensive validation of vesicle isolation is used including multiple protein markers. The authors also established key controls: buffer, buffer plus reagents, uEVs without reagents, and a lysed vesicle preparation to reduce false positives as well as "molecules of equivalent soluble fluorochrome" beads to standardize fluorescent intensity units. These key steps will allow for interlaboratory comparison of results regardless of instrument or software.

The second approach to uEV diagnostics is bulk assessment of uEV content. A seminal study by Pisitkun *et al.*[5]

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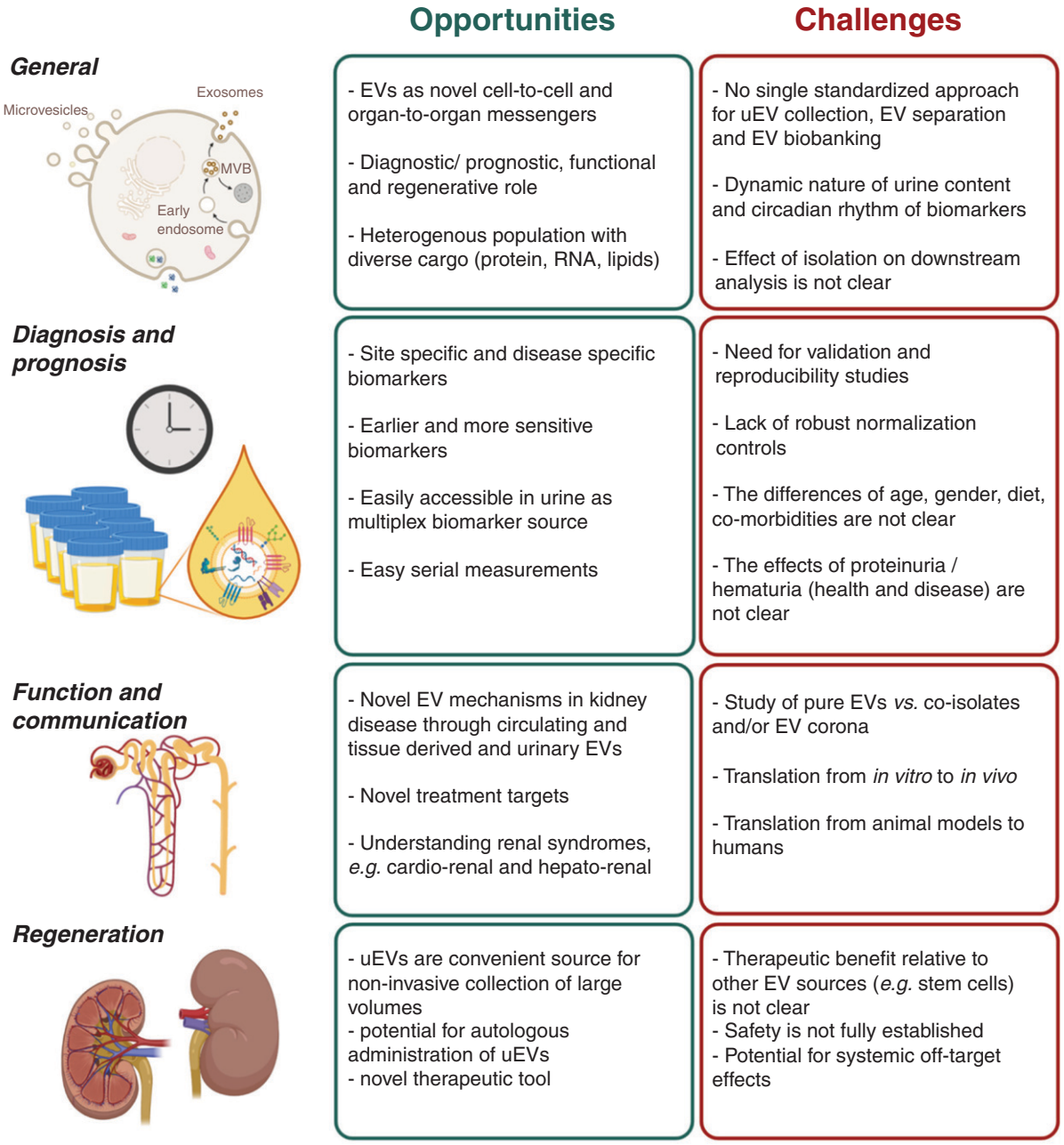


**Figure 1. Opportunities and challenges of uEVs in nephrology.** Recognizing opportunities and challenges will unravel the many sides of uEVs in nephrology and further mechanistic understanding and clinical application.

from 2004 established the feasibility of proteomic assessment of uEVs and identified fundamental challenges, such as the interference of Tamm–Horsfall protein. Similar approaches of EV cargo assessment have been described for EV-bound nucleic acids, lipids, and metabolites. A key assumption when inferring changes to the kidney from those seen in uEVs is that the molecular composition of uEVs mirrors those of the kidney. Recent work by Wu *et al.*[6] appears to support this. Using a proteomic approach, the authors showed strong correlations between uEV proteins and those found in whole kidneys. Changes to protein expression in whole kidneys following a physiologic stimulation (high $K^+$) were reflected in uEVs providing strong support for the use of uEVs as surrogate measures of kidney pathology.

## uEVs FOR FUNCTION AND REGENERATION

There is increasing interest in the functional and regenerative role of uEVs. Cells are able to communicate by releasing EVs, which modulate processes in recipient cells.[7–9] Three types of such communication have been described in the kidney: (*1*) intranephron communication that may explain

**Table 1.** Available resources for EV research

| Resource | Description | Website Location |
|---|---|---|
| | **EV molecular databases** | |
| Exocarta/Vesiclepedia | Collection of molecular data of EVs from multiple sources | http://www.exocarta.org/; http://www.microvesicles.org/ |
| exoRBase | Repository of EVs long RNAs (mRNA, lncRNA, and circRNA) from RNA-seq analyses in several human body fluids | http://www.exoRBase.org |
| exRNA Atlas | Repository of the Extracellular RNA Communication Consortium including small RNA sequencing and qPCR-derived exRNA profiles from human and mouse biofluids | https://www.exrna-atlas.org/ |
| | **Guidelines/position papers** | |
| MISEV 2018 | This position statement provides guidance in standardization of protocols and reporting in the EV field | https://www.pubmed.ncbi.nlm.nih.gov/30637094/ |
| uEVs | This is a position paper about uEVs by the Urine Task Force of ISEV | https://www.onlinelibrary.wiley.com/doi/10.1002/jev2.12093 |
| Blood EVs | This paper presents considerations toward a road map for collection, handling, and storage of blood EVs | https://www.tandfonline.com/doi/full/10.1080/20013078.2019.1647027 |
| EV RNA | This position paper presents the obstacles and opportunities in the functional analysis of EV RNA | https://www.tandfonline.com/doi/full/10.1080/20013078.2017.1286095 |
| EVs in therapy | This position paper discuss the application of EV-based therapeutics in clinical trials | https://www.tandfonline.com/doi/full/10.3402/jev.v4.30087 |
| | **Reporting** | |
| EV-TRACK platform | Platform for recording of experimental parameters of EV-related studies | https://www.evtrack.org/ |
| MIFlowCyt-EV | Framework for standardized reporting of EV flow cytometry experiments | https://www.tandfonline.com/doi/full/10.1080/20013078.2020.1713526 |
| | **Courses** | |
| Basics of Extracellular Vesicles | This MOOC provides basic knowledge about EVs | https://www.coursera.org/learn/extracellular-vesicles |
| Extracellular Vesicles in Health and Disease | This MOOC provides the current understanding about EVs and their role in health and diseases | https://www.coursera.org/learn/extracellular-vesicles-health-disease |
| Extracellular Vesicles: From Biology to Biomedical Applications | This practical course organized by EMBO covers different EV purification and characterization techniques and strategies to understand the role of EVs in biomedical applications | https://www.embl.org/about/info/course-and-conference-office/events/exo22-01/ |

mRNA, messenger RNA; lncRNA, long noncoding RNA; circRNA, circular RNA; RNA-seq, RNA sequencing; qPCR, quantitative PCR; exRNA, extracellular RNA; MISEV, minimal information for studies of extracellular vesicles; ISEV, International Society for Extracellular Vesicles; MOOC, massive open online course; EMBO, European Molecular Biology Organization.

how glomerular (*e.g.*, podocyte damage with proteinuria) and tubular damage (*e.g.*, hypoxia) causes interstitial fibrosis,[9] (*2*) intratubular communication (between tubular segments),[7] and (*3*) circulation to kidney communication, which was demonstrated in patients with active vasculitis and shown to bear B1-kinin receptors.[8] Understanding uEV-derived signaling pathways could ultimately lead to new therapeutic targets in renal disease. However, for functional studies, it is important to study pure EV preparations and contrast those fractions with coisolated non-EV material ("EV corona" or non-EV eluate).[1]

Researchers are starting to translate the *in vitro* findings to *in vivo* models, including use of labeled or tagged EVs, and to validate findings in human cohorts. For example, Lv *et al.*[10] used a transwell culture system to demonstrate that tubular epithelial cells stimulated by albumin produce EVs containing the inflammatory cytokines C-C Motif Chemokine Ligand 2 (CCL2). EVs from these albumin-treated tubular epithelial cells were injected into mice and induced tubular injury in an *in vivo* model. In addition, CCL2 messenger RNA in uEVs was found in patients with proteinuric IgA nephropathy, supporting the translational potential.[10]

The therapeutic role of stem cell–derived EVs has long been studied in acute kidney disease and CKD. Very recently, the regenerative role of uEVs from healthy donors was demonstrated

in a glycerol-induced AKI model.[11] uEVs improved renal recovery, stimulated tubular cell proliferation, and reduced expression of inflammatory and injury markers, restoring endogenous Klotho loss. The authors performed extra purification steps to obtain pure EVs, thorough EV characterization of EV (CD81, CD63), and non-EV–related proteins (calreticulin). The authors also included several key controls, including non-EV fractions and EVs from nonrenal sources.

## FUTURE DIRECTIONS

uEVs have been studied for >17 years since the hallmark publication by Pisitkun *et al.*[5] in 2004. Why is interest in uEVs as novel players in kidney disease ever increasing? In fact, 2004 launched an era of increasing rigor in uEV studies, where the field progressively overcame key roadblocks to clinical application. Biology and cargo loading of EVs were established; new isolation methods were developed, leading to purer EV isolates.[1] Quality markers were developed, normalization methods of uEVs were researched and validated,[12] and minimal reporting requirements were put in place (reviewed in ref. 1). Meanwhile, uEV research has ever increased because of the promises that uEVs hold. Most importantly, uEVs are enriched "baskets" of information on molecular processes and pathways that can be traced back to one cell type. Thus, they are potentially more sensitive than secreted proteins or RNA in urine, and they may also be more specific. Indeed, several attempts have been made to study EVs specific to certain tubule segments. However, this remains challenging, as many protein markers recognize only intracellular epitopes, which may necessitate permeabilization of uEVs by detergents.[12] A list of proteins that may be used for this purpose was outlined in the recently published uEV position paper.[1] Although the isolation of uEVs is still very time consuming and labor intensive, high-throughput uEV characterization methods are being developed and characterized[12] with the potential to speed up the process of getting kidney biomarkers clinically applicable, such as markers of transplant rejection that could bypass the need for kidney biopsy.[13]

Many of these advancements have increased the complexity of information that can be retrieved from uEVs. Therefore, the current challenges have shifted from finding isolation and characterization methods sensitive enough to study these novel messengers to improving specificity by (further) optimizing normalization methods, quality control, and thorough reporting. Here, we extend recommendations from the uEV position paper[1] with a selected collection of resources for uEV research (Table 1). Ultimately, addressing these challenges will lead to the fast and accurate methods with low variation necessary for clinical application. These next steps could make it possible for uEV-based approaches to replace kidney biopsies within the next decade.

## DISCLOSURES

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## AUTHOR CONTRIBUTIONS

C.J. Blijdorp, D. Burger, U. Erdbrügger, A. Llorente, and E.S. Martens-Uzunova conceptualized the study; U. Erdbrügger was responsible for visualization; C.J. Blijdorp, D. Burger, and U. Erdbrügger wrote the original draft; and C.J. Blijdorp, D. Burger, U. Erdbrügger, A. Llorente, and E.S. Martens-Uzunova reviewed and edited the manuscript.

## REFERENCES

1. Erdbrügger U, Blijdorp CJ, Bijnsdorp IV, Borràs FE, Burger D, Bussolati B, et al.: Urinary extracellular vesicles: A position paper by the Urine Task Force of the International Society for Extracellular Vesicles. *J Extracell Vesicles* 10: e12093, 2021
2. Burger D, Thibodeau JF, Holterman CE, Burns KD, Touyz RM, Kennedy CR: Urinary podocyte microparticles identify prealbuminuric diabetic glomerular injury. *J Am Soc Nephrol* 25: 1401–1407, 2014
3. Gilani SI, Anderson UD, Jayachandran M, Weissgerber TL, Zand L, White WM, et al.: Urinary extracellular vesicles of podocyte origin and renal injury in preeclampsia. *J Am Soc Nephrol* 28: 3363–3372, 2017
4. Musante L, Bontha SV, La Salvia S, Fernandez-Piñeros A, Lannigan J, Le TH, et al.: Rigorous characterization of urinary extracellular vesicles (uEVs) in the low centrifugation pellet - a neglected source for uEVs. *Sci Rep* 10: 3701, 2020
5. Pisitkun T, Shen RF, Knepper MA: Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A* 101: 13368–13373, 2004
6. Wu Q, Poulsen SB, Murali SK, Grimm PR, Su XT, Delpire E, et al.: Large-scale proteomic assessment of urinary extracellular vesicles highlights their reliability in reflecting protein changes in the kidney. *J Am Soc Nephrol* 32: 2195–2209, 2021
7. Oosthuyzen W, Scullion KM, Ivy JR, Morrison EE, Hunter RW, Starkey Lewis PJ, et al.: Vasopressin regulates extracellular vesicle uptake by kidney collecting duct

cells. *J Am Soc Nephrol* 27: 3345–3355, 2016
8. Kahn R, Mossberg M, Ståhl AL, Johansson K, Lopatko Lindman I, Heijl C, et al.: Microvesicle transfer of kinin B1-receptors is a novel inflammatory mechanism in vasculitis. *Kidney Int* 91: 96–105, 2017
9. Munkonda MN, Akbari S, Landry C, Sun S, Xiao F, Turner M, et al.: Podocyte-derived microparticles promote proximal tubule fibrotic signaling via p38 MAPK and CD36. *J Extracell Vesicles* 7: 1432206, 2018
10. Lv LL, Feng Y, Wen Y, Wu WJ, Ni HF, Li ZL, et al.: Exosomal CCL2 from tubular epithelial cells is critical for albumin-induced tubulointerstitial inflammation. *J Am Soc Nephrol* 29: 919–935, 2018
11. Grange C, Papadimitriou E, Dimuccio V, Pastorino C, Molina J, O'Kelly R, et al.: Urinary extracellular vesicles carrying klotho improve the recovery of renal function in an acute tubular injury model. *Mol Ther* 28: 490–502, 2020
12. Blijdorp CJ, Tutakhel OAZ, Hartjes TA, van den Bosch TPP, van Heugten MH, Rigalli JP, et al.: Comparing approaches to normalize, quantify, and characterize urinary extracellular vesicles. *J Am Soc Nephrol* 32: 1210–1226, 2021
13. Carreras-Planella L, Juega J, Taco O, Cañas L, Franquesa M, Lauzurica R, et al.: Proteomic characterization of urinary extracellular vesicles from kidney-transplanted patients treated with calcineurin inhibitors. *Int J Mol Sci* 21: 7569, 2020