# Extracellular vesicles in renal disease

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Abstract | Extracellular vesicles, such as exosomes and microvesicles, are host cell-derived packages of information that allow cell–cell communication and enable cells to rid themselves of unwanted substances. The release and uptake of extracellular vesicles has important physiological functions and may also contribute to the development and propagation of inflammatory, vascular, malignant, infectious and neurodegenerative diseases. This Review describes the different types of extracellular vesicles, how they are detected and the mechanisms by which they communicate with cells and transfer information. We also describe their physiological functions in cellular interactions, such as in thrombosis, immune modulation, cell proliferation, tissue regeneration and matrix modulation, with an emphasis on renal processes. We discuss how the detection of extracellular vesicles could be utilized as biomarkers of renal disease and how they might contribute to disease processes in the kidney, such as in acute kidney injury, chronic kidney disease, renal transplantation, thrombotic microangiopathies, vasculitides, IgA nephropathy, nephrotic syndrome, urinary tract infection, cystic kidney disease and tubulopathies. Finally, we consider how the release or uptake of extracellular vesicles can be blocked, as well as the associated benefits and risks, and how extracellular vesicles might be used to treat renal diseases by delivering therapeutics to specific cells.

Small membranous blebs or vesicles are released from the surface of most cells as a result of a variety of biological processes (FIG. 1). The smallest extracellular vesicles, called exosomes, are formed by the fusion of intracellular multivesicular bodies (also known as late endosomes) with the plasma membrane, leading to the release of their vesicular contents into the extracellular space[1]. Microvesicles are shed directly from the plasma membrane[2], whereas apoptotic bodies are the product of cellular degradation during programmed cell death (FIG. 1). This Review focuses on the biological functions of extracellular vesicles, specifically exosomes and microvesicles, and their contribution to renal diseases.

One of the most important functions of vesicle release is to rid the cell of unwanted substances. For example, activation of the complement system on host cells is followed by the release of complement-coated microvesicles that undergo phagocytosis; this function might protect the parent cell from complement-mediated cytolysis[3,4]. However, emerging data show that extracellular vesicles can transfer a multitude of receptors, proteins, genetic material (including mRNA and microRNA (miRNA)) and lipids, and thus shuttle information to cells in the immediate vicinity of, or at a distance from, the parent cell[5]. The transfer of RNA and miRNA can genetically reprogramme the recipient cells and thus alter their phenotype[6–8].

The content of extracellular vesicles can be located on their membranous surface and within the vesicle. As extracellular vesicles are released from lipid raft-enriched domains of the cellular membrane[9], the outer layer of the extracellular vesicle may have a higher concentration of receptors and membrane proteins than the parent cell, as has been demonstrated for microvesicles derived from platelets, monocytes and neutrophils[10–12]. The interaction between extracellular vesicles and recipient cells could involve the release of ligands from the extracellular vesicle that bind to receptors on the recipient cell, or direct binding between a ligand on, or within, the membrane of the extracellular vesicle and its receptor on the target cell. Alternatively, the extracellular vesicle membrane could fuse with the recipient cell membrane, or the entire extracellular vesicle could be taken up by the recipient cell by endocytosis[13] (FIG. 1). Importantly, the recipient cell may differ from the parent cell; for example, platelet-derived microvesicles can be taken up by endothelial cells[14] and monocytic microvesicles can fuse with platelets[10].

Extracellular vesicles have been detected in blood[14] and urine[15], as well as in other body fluids such as prostatic secretions[16], seminal fluid[17], cerebral spinal fluid[18], synovial fluid[19], breast milk[20], saliva[21], bile[22], ascites[23], amniotic fluid[15] and pleural fluid[1,24]. Their normal physiological functions may include immune modulation,

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**Key points**

- Extracellular vesicles are involved in cell–to–cell communication and they transfer nucleic acids, proteins and lipids that can alter the phenotype of the recipient cell
- Extracellular vesicles can be used as biomarkers of renal diseases
- Circulating microvesicles and exosomes may contribute to the development of renal diseases by immunomodulation, thrombogenesis and matrix modulation
- Extracellular vesicles may have a therapeutic role in tissue regeneration after acute kidney injury
- Extracellular vesicles have the potential to transfer endogenous and exogenous therapeutic substances to recipient cells

promotion of angiogenesis, adhesion, proliferation and tissue regeneration; their role in these processes will be discussed with special reference to kidney function. When extracellular vesicle release is enhanced, such as during disease processes, they may serve as biomarkers of particular diseases due to the presence of a specific antigenic profile. Furthermore, the role of extracellular vesicles in physiological processes in the resting state may become pathological when they are excessively released, such as during inflammation or malignancy. Disease-related processes, specifically renal diseases, will be elaborated on in this Review, as will potential treatments to block the excessive release of microvesicles. Moreover, we will discuss the capacity of extracellular vesicles to deliver their content to specific cells, which may be utilized to treat kidney disorders.

## Generation and cellular uptake of vesicles

Extracellular vesicles are released from resting cells, particularly during cell growth[7]. Vesicle release is markedly increased during cellular activation and/or cell stress, for example, in response to exposure to cytokines and pro-inflammatory stimulants, including tumour necrosis factor (TNF) and thrombin[25–27], bacterial toxins or viral components[13,14] and uraemic toxins[28]. In addition, the stimulation of purinergic receptors[29–31], low sheer stress[32], hypoxia[33] and cellular injury and senescence[34] can induce the release of extracellular vesicles.

Extracellular vesicles are defined by their mechanism of release and by the cellular markers that they carry. Understanding how extracellular vesicles are generated, released and taken up is crucial for the development of treatments that take advantage of their beneficial properties while blocking their undesired effects.

Exosomes are generated from multivesicular bodies, which contain intraluminal vesicles[1]. Intraluminal vesicles are formed by components of the endosomal-sorting-complex-required-for-transport (ESCRT), which comprises several intracellular protein complexes, or by lipids and tetraspanins, acting separately or in concert[1,35,36]. Intraluminal vesicles form by inward budding of the endosomal membrane and contain proteins, lipids, RNAs and cytosol[1]. Mitochondrial DNA may also be incorporated[37]. The docking and fusion of multivesicular bodies to the cell membrane is mediated by specific RAB GTPases[1] and, when released from multivesicular bodies, the intraluminal vesicles become exosomes. Exosomes are defined by their size, which generally ranges from 30 nm to 100 nm (although sizes up to 150 nm have been reported depending on the technique used to demonstrate them[1]), and by their content. As exosomes originate from endosomes, they will inherently contain endosomal molecules such as the tetraspanin CD63 and ESCRT components[38]. However, as shed microvesicles may also contain these components, exosomes cannot be differentiated from microvesiclegon8383s based solely on endosomal content[39].

Microvesicles are usually larger than exosomes (ranging from 100 nm to 1 μm in size) and are shed from cells by a regulated process that leads to the outward budding of the plasma membrane[40]. This process is primarily initiated by calcium influx into the cell as well as release of intracellular calcium[41], possibly amplified by enhanced mitochondrial membrane permeability, followed by actin cytoskeleton rearrangements associated with activation of calpains[2] and of the small GTPase ADP-ribosylation factor 6 (ARF6)[42], membrane remodelling with loss of phospholipid asymmetry, phosphatidylserine exposure and ultimately budding off of the vesicle. In resting cells, lipid asymmetry is maintained when phosphatidylserine and phosphatidylethanolamine are located on the inner leaflet of the cell membrane while phosphatidylcholine and sphingomyelin are located on the outer leaflet[43]. This asymmetry is governed by the activity of three enzymes: flippase (which directs the movement of lipids towards the inner leaflet), floppase (which directs the movement of lipids towards the outer leaflet) and scramblase (which has bidirectional activity)[43]. Disruption of membrane asymmetry is caused by calcium-mediated activation of floppase and scramblase and inhibition of flippase. These actions result in exposure of phosphatidylserine on the outer leaflet, present on many, but not all, microvesicle membranes. Phosphatidylserine can be readily detected as it binds to annexin V. Investigations of Scott syndrome, a rare bleeding disorder with reduced platelet-derived microvesicles, show dysfunctional scramblase activity[44], which could be due to a mutation in the transmembrane protein TMEM16F that has been demonstrated to have scramblase activity[45]. These findings could be reproduced in mice deficient in TMEM16F[46,47].

The properties and cargo of exosomes and microvesicles have been summarized in databases that are continuously updated, namely Vesiclepedia, ExoCarta and EVpedia[48]. The content incorporated in released extracellular vesicles is not random[24] and may be related to the stimulus inducing extracellular vesicle release as well as the cellular microenvironment[5,49,50], with enrichment of protein[51] and lipid[52] content within lipid rafts. Interestingly, the same cell may release extracellular vesicles that differ in the content of their membrane lipid composition and in their intravesicular cargo, which could reflect their pathway of biogenesis and the function for which they are destined, as has been demonstrated for extracellular vesicles secreted from mesenchymal stem cells (MSCs)[49].

Extracellular vesicles have a short half-life, which ranges from minutes up to 5.5 h after their release into the circulation[53]. This short half-life is most likely due

| | Exosomes | Microvesicles | Apoptotic bodies |
|---|---|---|---|
| **Formation** | Endosomal pathway, internal budding, exocytosis | Budding off the plasma membrane | Cell fragmentation/ blebbing |
| **Size** | 30–100 nm | 100–1,000 nm | 1–5 μm |
| **Content** | Proteins, lipids, mRNA, miRNA and cytosol | Proteins, lipids, mRNA, miRNA and cytosol | Proteins, lipids, DNA, rRNA, organelles and cytosol |

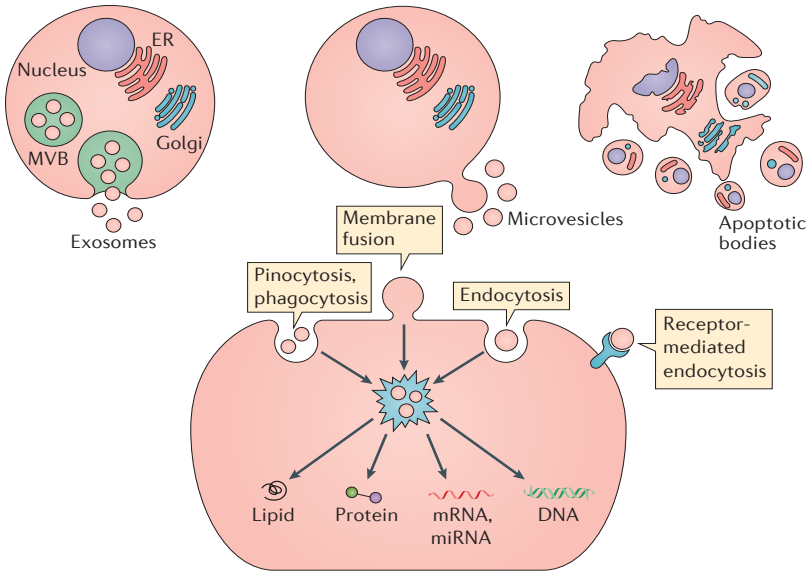


Figure 1 | **Release and uptake mechanisms of extracellular vesicles.** Extracellular vesicles can be classed as exosomes, microvesicles and apoptotic bodies, based on the mechanism by which they are released from cells and differentiated based on their size and content. Extracellular vesicles are taken up by cells by endocytosis, phagocytosis, pinocytosis, or membrane fusion. The ligands on the membrane of extracellular vesicles may also bind to receptors on recipient cells or be released from the extracellular vesicle and bind to a receptor on target cells to thereby induce an intracellular signal. ER, endoplasmic reticulum; miRNA, microRNA; MVB; multivesicular body; rRNA, ribosomal RNA.

to uptake by cells. Uptake by neighbouring or distant cells occurs by various endocytic mechanisms, including clathrin-dependent, clathrin-independent and caveolin-mediated endocytosis, as well as by macropinocytosis, phagocytosis and lipid raft-mediated internalization[54] (FIG. 1). Membrane fusion with the cell membrane of target cells may also occur. The endocytic pathway that is chosen depends on proteins, proteoglycans and lipids that are present on the surface of extracellular vesicles and recipient cells, as well as on mediators in the extracellular microenvironment.

## Detection of extracellular vesicles

In order to characterize extracellular vesicles, they need to be isolated and purified or identified within bodily fluids or tissues. The most abundant extracellular vesicles in blood are derived from platelets[55], but extracellular vesicles from other blood cells (leukocytes and red blood cells) and endothelial cells are detectable even in healthy individuals[14,56,57]. In urine, the most abundant extracellular vesicles are derived from cells that line the tubular lumen and from podocytes[58–62].

Extracellular vesicles may be identified within tissues and fluids based on their size and cellular markers using flow cytometry or electron microscopy with conjugated antibodies. Methodology used for the isolation of extracellular vesicles and for assaying their content includes differential centrifugation followed by flow cytometry, resistive pulse sensing, capture-based assays, electron or atomic force microscopy, nanoparticle tracking analysis, dynamic light scattering or proteomics[60,61,63–65]. Specific methodology has been developed for the detection of extracellular vesicles in urine[60,66–68], particularly as these may be entrapped within polymers of Tamm–Horsfall protein (also known as uromodulin)[24,69,70].

## Vesicle interactions with cells

Extracellular vesicles may transport their cargo to neighbouring or remote cells, thus altering the properties of recipient cells. The incorporation of proteins, lipids and nucleic acids into extracellular vesicles, and the uptake of extracellular vesicles by target cells, occurs in a series of steps. Proteins, mRNAs and miRNAs can be packaged and sorted by signals within the extracellular vesicle and the cellular and extracellular microenvironment, so as to promote their ultimate function[71]. For example, delivery of membrane-type 1 matrix metalloproteinase (MT1-MMP) is mediated by vesicle–associated membrane protein 3 (VAMP3) in tumour-derived microvesicles, thus enhancing invasive potential[72]. Invasive properties may explain how extracellular vesicles not only release their cargo in target cells but are also capable of passing through the cells. Extracellular vesicles may be capable of transferring through cells by transcytosis[29], and have been shown to pass through basement membranes[14].

The lipid content of exosomes may differ from that of their parent cells[73], with enrichment of phosphatidylethanolamine and altered membrane structures. Similarly, nucleic acids may be selectively incorporated into extracellular vesicles by specific 'zip-code-like' signals, as shown for microvesicles derived from glioblastoma cells in which sequences in the 3′ untranslated regions of mRNA, incorporating a miRNA-binding site, targeted mRNA into microvesicles[74]. Extracellular TNF affects the miRNA content of extracellular vesicles from endothelial cells and their capacity to be taken up and to transfer their cargo to target cells[75]. Also, the pH in the vicinity of extracellular vesicles can affect their uptake[76]. The homing and uptake of extracellular vesicles may be mediated by signals on the recipient cell and/or binding between ligands on extracellular vesicles and receptors on recipient cells[77]. For example, in endothelial cells, uptake of platelet-derived microvesicles was regulated by the presence of a phosphatidylserine-binding opsonin called developmental endothelial locus 1 (DEL1)[78].

mRNAs and miRNAs present in extracellular vesicles are functional and, after uptake by recipient cells, can be translated[6], even between cells originating from different species (for example, mouse to human). The transfer of nucleic acids has the capacity to affect the recipient cell's phenotype and the transfer of RNA is not random. For example, specific miRNAs within microvesicles derived

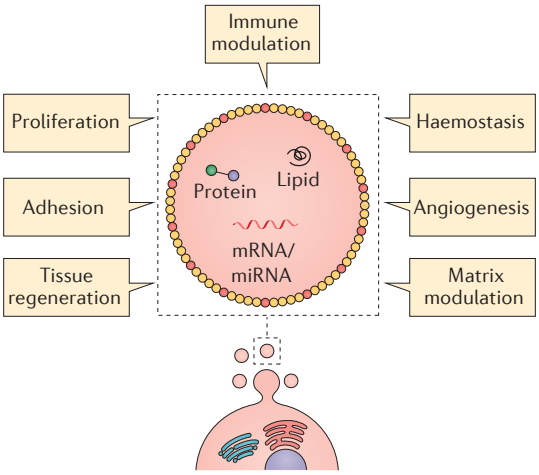


Figure 2 | **Physiological processes influenced by extracellular vesicles.** The lipid bilayer contains phosphatidylserine on the outer leaflet (pink circles) due to a loss of membrane asymmetry. The content of extracellular vesicles, including mRNAs, microRNAs (miRNAs), lipids and proteins, are depicted.

from human adult MSCs were shown to be transferred to murine tubular epithelial cells[79]. Similarly, organ-specific extracellular vesicles that were released from lung or liver cells induced mRNA expression in bone marrow cells[80]. B cells infected with Epstein–Barr virus released exosomes containing viral miRNAs that were capable of silencing genes in recipient monocyte-derived dendritic cells[81].

In addition to RNAs, tumour microvesicles may carry single-stranded and double-stranded DNA[82–84], although the functional consequence of horizontal DNA transfer is unknown. The presence of DNA in extracellular vesicles may also be due to contamination, as demonstrated for urinary extracellular vesicles that were studied as biomarkers of renal disease[85]. In addition to DNA, histones have been detected within extracellular vesicles[86]. Studies have also demonstrated the presence of mitochondrial DNA within exosomes[37], with some reports suggesting that mitochondria themselves may be transferred between cells via extracellular vesicles[87,88] or in CD63-positive particles[89].

Extracellular vesicles can transport and transfer multiple proteins simultaneously[9], some of which would not be possible in soluble form or would be quickly removed by the adaptive immune response if circulating in free form. Thus, the transfer of proteins within extracellular vesicles may allow cargo to evade the immune response. Importantly, extracellular vesicles can transfer fully functional receptors from one cell type to another, allowing cell signalling to occur in cells that originally lacked the receptor; this has been shown for C-C chemokine receptor type 5 (CCR5), epidermal growth factor receptor (EGFR), C-X-C chemokine receptor type 4 (CXCR4), glycoprotein IIb/IIIa (also known as integrin αIIbβ3 and CD41) and the kinin B1 receptor[90–95]. The transfer of platelet-derived receptors and antigens to haematopoietic stem cells was shown to have a role in the homing of these cells[96]. In addition to protein transfer, the shuttling of lipids via extracellular vesicles seems to have an important role in cell signalling and cell activation[97]. Furthermore, even bacterial toxins[14], lipid components of mycobacteria[98] and viral components[13,99,100] are transported by host extracellular vesicles and released within vesicles.

## Extracellular vesicles in physiological processes

Extracellular vesicles are involved in most physiological processes that are associated with intercellular communication. These processes, such as immune modulation, haemostasis, vessel integrity and tissue regeneration, affect the development and function of organs and systems, including the kidney (FIG. 2).

***Immune modulation.*** Exosomes that are secreted from tumours or from dendritic cells may have antigen-presenting properties and may carry major histocompatibility complex (MHC)–peptide complexes that enable them to activate T cells and dendritic cells[101]. Extracellular vesicles can promote and suppress immune responses (reviewed elsewhere[101]). For example, extracellular vesicles derived from polymorphonuclear leukocytes may modulate immune responses by stimulating the release of pro-inflammatory cytokines[102] but also modulate immune responses through the release of anti-inflammatory mediators[103,104]. Most studies of the immunomodulatory effects have been carried out using extracellular vesicles released from stem cells and tumour cells, and demonstrated the immunosuppressive effects of transcription factors and miRNAs (reviewed elsewhere[105]). These properties could potentially be utilized to suppress allograft rejection, as has been shown using exosomes derived from bone marrow dendritic cells given to rats receiving heart allografts[106], and to block inflammatory responses during sepsis[107]. Similarly, platelet-derived microvesicles obtained after storage downregulated macrophage reactivity and dendritic cell differentiation[108], although it is not clear if these properties correspond to the *in vivo* setting.

In addition to affecting the innate immune response, platelet-derived microvesicles can also modulate adaptive immune responses by delivering CD40 ligand (also known as CD154) to germinal centres, leading to the proliferation of B cells and the production of IgG[109]. Microvesicles can also alter the endothelium to a more reactive state, such as has been demonstrated using microvesicles derived from lipopolysaccharide-stimulated platelets bearing IL-1β, which have been shown to induce the production of endothelial vascular cell adhesion protein 1 (VCAM1) *in vitro*[110]. Consequently, platelet-derived microvesicles promote the adhesion of monocytes to the endothelium[111]. Such an interaction has the potential to induce an inflammatory state.

Interestingly, microvesicles that are released from platelets and endothelial cells possess chemoattractant properties. Platelet-derived microvesicles were shown to induce chemotaxis of haematopoietic cell lines as well as bone marrow-derived CD34-positive cells[93] and induce monocyte recruitment to endothelial cells by deposition

of RANTES[112]. Endothelial cell-derived microvesicles attracted neutrophils due to the presence of the kinin B1 receptor and IL-8 (REF. 57).

Complement activation is usually directed towards foreign cells, such as bacteria, or unwanted host cells, for example, during apoptosis. Activation of the terminal complement complex (composed of C5b–9) on platelets or red blood cells causes these cells to release microvesicles[4,113]. Complement is deposited on microvesicles derived from platelets, leukocytes and red blood cells[56,108,114,115], either due to complement activation on the parent cells or directly on the microvesicles. This phenomenon would be expected to occur predominantly during complement-mediated disease, and minimally during health. Blood cell-derived microvesicles expose complement regulators, such as complement receptor 1 (CR1), decay accelerating factor (DAF; also known as CD55), CD59 and membrane cofactor protein (MCP; also known as CD46)[3,116], which would prevent excessive complement activation from occurring on the microvesicle under physiological conditions. Complement-coated blood cell-derived microvesicles are readily phagocytosed by neutrophils[3], suggesting that the release of these microvesicles from blood cells may be cytoprotective.

***Haemostasis and platelet aggregation.*** Microvesicles contribute to coagulation, platelet aggregation and thrombosis. The main mechanisms for initiating coagulation and thrombosis are by exposing phosphatidylserine and tissue factor, respectively, on their surfaces. Phosphatidylserine flipping to the outer leaflet of the microvesicle creates a negatively charged surface with binding sites for prothrombin (coagulation factor II), factor Va, and factor Xa[41,117]. Tissue factor is normally encrypted, but when exposed, such as on a microvesicle, it initiates the extrinsic pathway of coagulation after binding to factor VIIa on surfaces containing phosphatidylserine, ultimately leading to the generation of thrombin and platelet clotting[9]. Tissue factor is expressed on monocytes and their microvesicles, which can fuse with platelets[10] and thus possibly transfer tissue factor to platelets. Monocyte-derived microvesicles can bind to platelets due to expression of P-selectin glycoprotein ligand 1 (PSGL-1) that binds to P-selectin on platelets[118]. Platelet-derived microvesicles may harbour tissue factor[119], possibly after transfer from monocytes. Microvesicles derived from endothelial cells were also shown to be thrombogenic via a tissue factor-dependent mechanism[120] and to be capable of transferring this prothrombotic property to monocytes[121].

Platelet-derived microvesicles are more procoagulant then the platelets themselves[11]. In addition to expressing tissue factor, platelet-derived microvesicles may be prothrombotic due to the presence of protein disulfide isomerase[122] and receptors for factor VIII[123], by activating the intrinsic pathway of coagulation via factor XIIa[124] or by metabolizing arachidonic acid to thromboxane A2 (REF. 97), contributing to platelet aggregation. As not all microvesicles expose phosphatidylserine on their exterior[125], these mechanisms may explain how phosphatidylserine-negative microvesicles contribute to platelet activation. Neutrophil-derived microvesicles can activate platelets by binding to them via integrin αMβ2 (also known as macrophage-1 antigen (Mac-1))[126]. In addition to the procoagulant effects described, microvesicles in the circulation may also have certain antithrombotic effects under physiological conditions[127,128].

***Vessel integrity and angiogenesis.*** Under resting conditions, the endothelial cell lining is maintained and platelets are not activated. Microvesicles in the circulation may promote low-grade thrombin generation[127]. Endothelial cell-derived microvesicles protect the vascular endothelial cell lining by removing caspase-3 (REF. 129) and by exposing protein C receptor, thereby binding activated protein C and promoting cell survival[130,131]. Potentially, removal of activated complement components from endothelial cells by the release of complement-coated microvesicles could also promote their survival. In the injured vasculature, platelet-derived microvesicles enhance the regeneration of endothelial cells and their adhesion to the extracellular matrix[5].

It is unclear if microvesicles contribute to angiogenesis under physiological conditions. In tumours, platelet, glioblastoma and colon cancer-derived extracellular vesicles can promote endothelial cell proliferation and angiogenesis[132,133]. Microvesicles derived from endothelial progenitor cells were shown to be taken up by endothelial cells and promoted endothelial cell survival and cell growth as well as the formation of capillary-like structures, suggesting that they activated an angiogenic programme[8]. Similar results were observed in studies using endothelial cell-derived microvesicles[134]. *In vitro* studies using platelet-derived microvesicles showed that these also promoted endothelial cell survival[135]. Growth factors within microvesicles, such as transforming growth factor β in leukocyte-derived extracellular vesicles and vascular endothelial growth factor (VEGF) in MSC-derived extracellular vesicles[103,136], may contribute to angiogenesis. Similar results were obtained *in vivo*[137], showing that platelet-derived extracellular vesicles have the potential to promote revascularization after ischaemic injury, which could be an important reparative mechanism in acute kidney injury (AKI).

***Matrix modulation and tissue regeneration.*** Extracellular vesicles may restore and remodel cells and stroma after injury. This remodelling may occur through the uptake of extracellular vesicles by stem cells and early progenitor cells and the release of specific receptors, signals or RNAs from the vesicles (reviewed elsewhere[138]). Extracellular vesicles from lung epithelial cells altered the phenotype of bone marrow cells by horizontal RNA transfer, and these cells could be used to repair lung injury[139]. Extracellular vesicles released from stem cells may also beneficially affect injured organ cells, and these properties have therapeutic potential (see below).

Extracellular vesicles may have invasive potential, as shown for tumour-derived microvesicles[140] that may contain matrix metalloproteinases (MMPs) such as MMP2 and MMP9 or MT1-MMP[141,142], thus contributing to

tumour spreading. Exosomes may carry heat shock protein 90 (HSP90), which can activate MMP2 and plasmin to promote cancer cell invasion[143].

***Nephron function.*** Extracellular vesicles can pass from the systemic circulation into endothelial cells and tubular epithelial kidney cells[144] and into the urine[145,146]. The uptake of extracellular vesicles by kidney collecting duct cells is regulated by vasopressin[145]. The capacity to transfer from the circulation to the urine suggests that extracellular vesicles can pass through basement membranes[14] under both physiological and pathological conditions. In addition to originating in the circulation, extracellular vesicles in the urine may originate from tubular epithelial cells and glomerular cells[58–62] and carry a multitude of proteins[24,147] and nucleic acids[59]. An important function of their release is likely to rid cells of unnecessary or damaging components. Studies have shown, however, that exosomes can transfer signals between mCCDC11 murine kidney collecting duct cells (REF. 148), leading to functional aquaporin 2 in recipient cells; this observation suggests that urinary extracellular vesicles can transfer information between neighbouring cells, or downstream, thus affecting physiological tubular function[24,60]. Extracellular vesicles in urine may be transported or entrapped within polymers of uromodulin[60] and it has been speculated that this large protein may modulate the interaction between extracellular vesicles and their target cells along tubular lumina[24]. Furthermore, multipotent progenitor cells in glomeruli or tubuli have regenerative capacity[149]. Extracellular vesicles released from resident glomerular MSCs stimulated tubular cell regeneration in a model of AKI[150].

In healthy living donors, urinary extracellular vesicles derived from glomerular and tubular cells were correlated with nephron hypertrophy and nephrosclerosis, thus enabling extracellular vesicles in urine samples to identify structural age-related changes in the kidneys in a non-invasive manner[151].

## Extracellular vesicles in renal diseases

Extracellular vesicles may be biomarkers of renal disease, as well as mediators of inflammation, thrombosis, adhesion, immune suppression, or growth and regeneration. Within the kidney, they can originate from blood cells, endothelial cells, podocytes or tubular epithelial cells, and they can be detected within the circulation, urine or tissue. Here, we describe the contributions of extracellular vesicles to various renal diseases.

***Thrombotic microangiopathies.*** Microvesicles have been detected in the major subtypes of thrombotic microangiopathies (TMA), including haemolytic uraemic syndrome induced by Shiga toxin-producing *Escherichia coli* (STEC-HUS), atypical haemolytic uraemic syndrome (aHUS) and thrombotic thrombocytopaenic purpura (TTP). In all forms of TMA, platelet activation and endothelial cell injury are predominant features, regardless of aetiology[152].

In STEC-HUS, elevated levels of microvesicles derived from platelets, monocytes, neutrophils and red blood cells have been detected[56,119,153]. Our group has shown that microvesicles, mostly from platelets and monocytes, are positive for tissue factor and bind to annexin V (via phosphatidylserine) during the acute phase of STEC-HUS. The presence of tissue factor and phosphatidylserine on the microvesicles most likely contributes to the formation of microthrombi during the acute phase[119]. In addition, platelet and monocyte-derived microvesicles contain deposits of C3 and C9 during the acute phase of disease, reflecting complement activation in the circulation[3] (FIG. 3). The levels of blood cell-derived microvesicles decrease after recovery from the disease[119].

Release of tissue factor-positive microvesicles could be reproduced *in vitro* when whole blood was stimulated with Shiga toxin or lipopolysaccharide from *E. coli* O157:H7, and even more so when blood was co-stimulated with both virulence factors. Furthermore, tissue factor was elevated in plasma when these experiments were carried out in a perfusion chamber[119]. *In vitro* experiments also showed that Shiga toxin induced the release of C3-positive and C9-positive microvesicles from platelets and monocytes[3] and the release of C9-positive microvesicles from red blood cells, and that toxin-induced complement activation on red blood cells leads to haemolysis, which is a major feature of HUS[56].

Microvesicles not only participated in thrombosis and haemolysis but were shown to transport active Shiga toxin into the kidneys, both to glomerular endothelial cells and to tubular epithelial cells via peritubular capillaries[14] (FIG. 3). As STEC are non-invasive bacteria, and only small amounts of free Shiga toxin are detected in the circulation[154], the circulation of toxin within microvesicles might explain how the toxin evades the immune response and is taken up by renal cells, leading to renal failure. Interestingly, these studies showed that microvesicles containing Shiga toxin can pass through renal cells and glomerular and tubular basement membranes[14]. This process could possibly be facilitated by toxin-induced kidney injury. Taken together, the receptor-mediated uptake of Shiga toxin by blood cells and the release of toxin within blood cell-derived microvesicles, which are also positive for tissue factor and complement, followed by the uptake of toxin-positive microvesicles by kidney cells, can explain the cardinal features of HUS: thrombocytopenia due to toxin-mediated platelet activation[155], tissue factor expression on microvesicles[119] and glomerular endothelial cell injury[156], haemolysis due to complement activation on red blood cells, and their fragmentation on injured endothelium, and renal failure due to toxin-induced kidney cell death[152].

aHUS is primarily associated with complement dysregulation, whether due to loss-of-function mutations in complement regulators, gain-of-function mutations in complement factors or autoantibodies against the regulatory component factor H, all of which enhance complement activation[152]. Mutations in factor H promote complement activation on platelets from patients with aHUS, the activation of platelets and their release of annexin V-binding-positive and tissue factor-positive microvesicles[114]; these events could be a mechanism of platelet activation and thrombosis in aHUS[157] (FIG. 3).

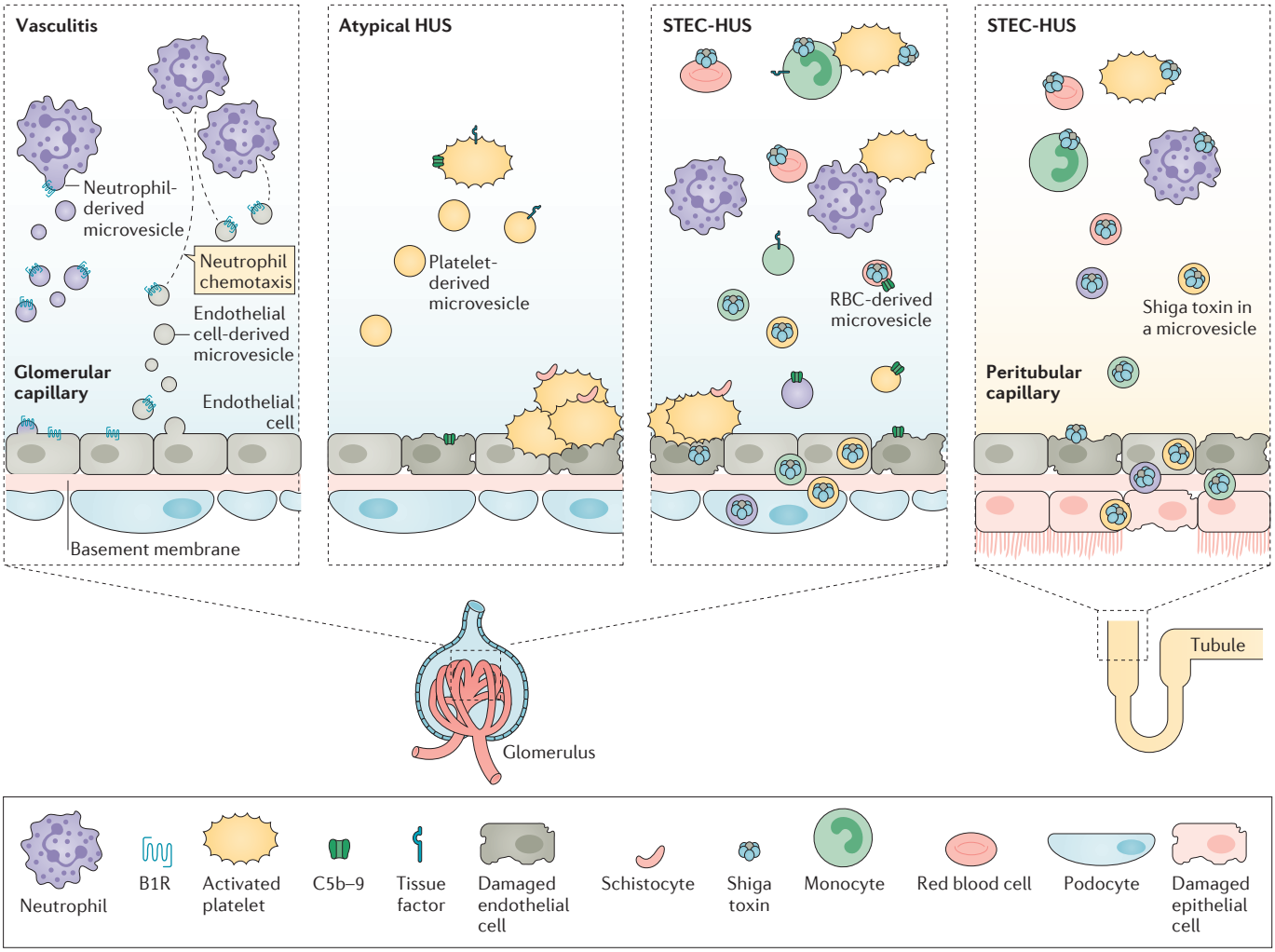


Figure 3 | **Involvement of microvesicles in vasculitis and thrombotic microangiopathies.** In vasculitis, the levels of neutrophil-derived and endothelial cell-derived microvesicles are elevated. Neutrophil-derived microvesicles can transfer functional kinin B1 receptor (B1R) to glomerular endothelial cells to activate an inflammatory signal. B1R on glomerular endothelial cell-derived microvesicles can induce neutrophil chemotaxis, further enhancing inflammation. In atypical haemolytic uraemic syndrome (HUS) caused by mutations in complement factor H, which cause dysfunctional complement regulation and enhanced complement activation, platelets are positive for complement components C3 and C9, suggesting formation of the terminal complement complex (C5b–9) on the platelets. Circulating microvesicles were thrombogenic, as they are positive for tissue factor. In Shiga toxin-producing *Escherichia coli* HUS (STEC-HUS), circulating blood cells form aggregates between platelets and leukocytes, and platelet–monocyte aggregates are thrombogenic, as they express tissue factor. Shiga toxin can circulate bound to blood cells, such as platelets, monocytes and neutrophils. Blood cell-derived microvesicles are tissue factor-positive and also exhibit deposits of complement C3 and C9. The microvesicles released from platelets and leukocytes transport Shiga toxin to renal glomerular endothelial cells and peritubular endothelial cells in which the toxin can induce its cytotoxic effect. Alternatively, toxin-positive microvesicles can transfer to podocytes and tubular epithelial cells through basement membranes. RBC, red blood cell.

TTP is a form of thrombotic microangiopathy associated with deficient or dysfunctional von Willebrand factor (VWF)-cleaving protease, due to mutations[158] or autoantibodies to the protease ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motifs 13). TTP is characterized by deposits of ultra-large multimers of VWF and the formation of microthrombi within capillaries that can obstruct blood flow in the glomeruli of the kidney[159] and in various other organs[160]. TTP was one of the first thrombotic conditions in which platelet-derived microvesicles were detected in the circulation and related to calpain activity[161]. Studies have shown that endothelial cell-derived microvesicles in the circulation of patients with TTP are procoagulant and proadhesive, as they contain CD62E (E-selectin), VWF, intercellular adhesion molecule 1 (ICAM-1), platelet endothelial cell adhesion molecule (PECAM-1; CD31) and endoglin (CD105)[162]. Our group has demonstrated that these extracellular vesicles carry complement C3 and C9 and that perfusion of plasma from patients with TTP over glomerular endothelial cells can induce complement deposition on shed endothelial

microvesicles, indicating that complement is activated on the endothelium during TTP[32] and thus promotes vascular injury.

***Vasculitides.*** Vasculitis is associated with the activation of neutrophils and with endothelial dysfunction. Children and adults with vasculitides have elevated levels of microvesicles that are derived from endothelial cells, neutrophils and platelets[163,164]. These microvesicles are positive for markers of cellular activation, correlate with the Birmingham vasculitis activity score and can be used to monitor disease activity[165]. Neutrophil-derived microvesicles reach much higher levels during acute vasculitis than in other nephropathies, reflecting the degree of neutrophil activation[166].

Anti-neutrophil cytoplasmic antibodies (ANCA) that circulate in certain vasculitides induce the release of microvesicles from neutrophils[167]. Neutrophil-derived microvesicles expose phosphatidylserine, selectins, integrins and, importantly in the context of vasculitis, even myeloperoxidase and proteinase 3 on their surface[167,168]. These enzymes were also detected in urinary microvesicles[169]. Microvesicles in the circulation during the acute phase of disease may be prothrombotic, as they expose tissue factor on their surface[170] and promote the generation of thrombin[167] and may, therefore, contribute to thromboembolic disease in vasculitis[171]. The generation of thrombin may be dependent on the presence of C5a in plasma and induced by ANCA[170]. Neutrophil-derived microvesicles bind to C1q[168], which could suggest that they can activate complement through the classical pathway. These microvesicles can bind to monocytes and endothelial cells[168] and induce endothelial cell activation and injury[167].

Our group has shown that the kinin–kallikrein contact system is activated in children and adults with vasculitis, thus promoting inflammation[172,173]. During sustained and chronic inflammation, kinins bind to the B1 receptor, which is exposed on the cell surface after ligand binding. Neutrophil-derived microvesicles that are released during vasculitis expose the B1 receptor and are capable of transferring functional B1 receptors to glomerular endothelial cells, thus promoting the inflammatory response[95,174]. Endothelial cell-derived microvesicles circulating during vasculitis are also B1 receptor-positive and we have shown that exposure of this receptor induces neutrophil chemotaxis[57] (FIG. 3). C1 inhibitor is the main inhibitor of the kinin–kallikrein system. Plasma from patients with vasculitis or C1 inhibitor-depleted plasma perfused over glomerular endothelial cells also induced the release of B1 receptor-positive microvesicles. Addition of C1 inhibitor decreased the release of B1 receptor-positive microvesicles from glomerular endothelial cells, an effect we suggested should be explored as a treatment for inflammation associated with kinin receptor-positive microvesicles[57].

***IgA nephropathy.*** In IgA nephropathy, a profile of elevated miRNAs derived mostly from red blood cells or their microvesicles were detected in urine[175]. The researchers speculated that these extracellular vesicles could be taken up by, and affect, renal parenchymal cells. Others have also correlated particular miRNA profiles with disease severity, although these miRNAs may come from cells that are passed into the urine rather than from vesicles[176]. Protein biomarkers in patient urinary exosomal fractions included α1-antitrypsin, aminopeptidase N, vasorin precursor, ceruloplasmin, and podocalyxin[69]. A proteomics study in a limited number of patients identified four proteins associated with urinary exosomes in early stages of the disease that could be used as biomarkers to distinguish IgA nephropathy from thin basement membrane disease (which, like IgA nephropathy, presents with recurrent haematuria) and from control samples[177].

***Nephrotic syndrome.*** Urine from patients with nephrotic-range proteinuria, regardless of its cause, contains large amounts of protein, which may affect the detection of extracellular vesicles and their proteins. Therefore, specific methodology has been developed for optimal isolation of urinary extracellular vesicles in nephrotic urine using ultracentrifugation followed by size-exclusion chromatography[68] or sucrose gradient density centrifugation[169]. Using a podocyte-derived microvesicle-enriched fraction of urine, proteomic analysis of samples from patients with nephrotic syndrome showed that they contained proteins associated with proteinuria, namely nephrin, transient receptor potential cation channel 6 (TRPC6), inverted formin 2 (INF2) and phospholipase A2 receptor[169]. Patients with focal segmental glomerulonephrosis and steroid-sensitive nephrotic syndrome had elevated levels of Wilms tumour 1 (WT1) in urinary exosomes, indicating podocyte involvement[59,178]. The presence and levels of WT1 could, however, not differentiate between different forms of childhood nephrotic syndrome[179]. Lysosome membrane protein 2 (LIMP2) was detected in urinary microvesicles from patients with membranous nephropathy and its presence correlated with findings in renal biopsy samples, as LIMP2 was upregulated in patient glomeruli, suggesting that urinary microvesicles could be used as biomarker of disease[180].

Few studies have investigated the presence and role of extracellular vesicles in the circulation of patients with nephrotic syndrome. In patients with membranous nephropathy and minimal change nephrotic syndrome, extracellular vesicles that are released from red blood cells, platelets and endothelial cells expose phosphatidylserine, which may contribute to thrombotic complications[181].

*In vitro* studies have shown that the incubation of endothelial and monocytic microvesicles with podocytes induces the secretion of pro-inflammatory mediators from the podocytes and decreases albumin endocytosis, thus potentially contributing to the development of proteinuria[182].

***Urinary tract infection, cystic kidney disease and tubulopathies.*** Patients with febrile urinary tract infections exhibited high levels of tissue factor activity in the microvesicle suspension extracted from plasma, correlating with disease severity. Tissue factor activity was highest in the plasma of patients with bacteraemia[183], suggesting that it may contribute to a prothrombotic state during

sepsis. Extracellular vesicles may protect against bacterial infection, as the proteome of urinary exosomes contained antibacterial proteins and peptides that can inhibit the growth, and promote the lysis of, *E. coli* strains; thus, they may function as an immunological barrier against urinary tract infections[184].

Urinary extracellular vesicles can be used to diagnose other kidney diseases based on their protein and miRNA profiles, to study the phenotype of aberrant proteins they carry and to differentiate between diseased and healthy conditions. This ability was exemplified in autosomal dominant polycystic kidney disease, in which polycystin 1 and polycystin 2 were reduced and transmembrane protein 2 (TMEM2) was increased in exosomes. The polycystin 1 or polycystin 2:TMEM2 ratios correlated inversely with kidney volume[185] and the vesicles and mutated proteins interacted with primary cilia of renal epithelial cells[186]. Likewise, a miRNA profile was distinguished in autosomal dominant polycystic kidney disease with decreased miR-1 and miR-133a (kidney tumour suppressors) in urinary extracellular vesicles[187]. Exosomes could also be used for diagnosing tubulopathies, as urinary exosomal fractions from patients with Gitelman syndrome and Bartter syndrome lacked the thiazide-sensitive Na–Cl co-transporter and the Na–K–Cl co-transporter 2, respectively[61,147,188].

***Acute kidney injury.*** In septic patients with AKI, platelet-derived extracellular vesicles were found to be elevated[189]. Circulating miRNAs were also elevated, and these miRNAs were not removed by dialysis filters[190]. Plasma obtained from patients with AKI and burns, when applied *in vitro* to podocytes and tubular epithelial cells, induced cytoskeletal and apoptotic alterations, which may partly explain how the patients developed proteinuria and renal failure[191,192]. These cellular alterations might be due to circulating cytokines, microvesicles and other soluble mediators that were removed by resin adsorption[191].

Urinary extracellular vesicles may also be used as biomarkers of AKI in patients with maintained diuresis. For example, levels of $Na^+/H^+$ exchanger isoform 3, a marker of tubular injury, were elevated in urinary membrane fractions from patients with acute tubular necrosis[193], compared with patients with prerenal azotaemia and controls. Urinary exosomal fetuin A was found in a limited number of intensive care unit patients with AKI[194]. Furthermore, a transcription factor, activating transcription factor 3 (ATF3), was identified as a biomarker of tubular injury in urinary exosomes from a limited number of patients with AKI, both at the protein[59] and RNA[195] level. Microvesicles from MSCs may have therapeutic potential in AKI (see below).

Several studies have attempted to correlate extracellular vesicle levels with the course of disease during sepsis. Patients with sepsis exhibited elevated levels of platelet-derived and endothelial cell-derived microvesicles, which enhanced vessel reactivity in an animal model[196]. An increase in endothelial cell-derived and leukocyte-derived microvesicles was observed in patients that developed disseminated intravascular coagulation, and the endothelial microvesicles were positive for endoglin and PECAM-1 (REF. 197). Endothelial extracellular vesicle elevation early in sepsis was associated with improved survival[198]. A comparable trend was found between high circulating extracellular vesicles and lower mortality[199]. Patients with severe multiorgan failure during sepsis had lower levels of platelet-derived extracellular vesicles and tissue factor-positive extracellular vesicles, but higher levels of granulocyte-derived and erythrocyte-derived extracellular vesicles[200]. By contrast, others found that the most severe cases of meningococcal septicaemia were associated with higher levels of circulating procoagulant extracellular vesicles[201].

Microvesicles have been demonstrated to have antibacterial activity. Patients with bacteraemia were reported to have elevated levels of neutrophil-derived microvesicles and these microvesicles possessed bacteriostatic properties[202] that prevented the growth of both *E. coli* and *Staphylococcus aureus*. Similarly, procoagulant microvesicles could bind to *Streptococcus pyogenes* and prevent its growth and dissemination[203]. However, it should be noted that the procoagulant and proinflammatory effects of microvesicles may also contribute to organ failure during sepsis, including in patients with AKI and in animal models[204–206].

***The effect of extracellular vesicles on tissue regeneration.*** MSCs can originate in the bone marrow, adipose tissue, cord blood, and also in the kidney[207]. MSCs have been reported to induce tissue regeneration after injury in numerous studies. The beneficial effects of MSCs may be mediated in a paracrine manner via the transfer of extracellular vesicles containing immune modulators to injured tissue. The regenerative potential of microvesicles has been evaluated in preclinical studies, using various organs including the heart, lungs and kidneys (reviewed elsewhere[208]). In rodent models of AKI, an improvement in renal parameters was documented[79,144,209]. Furthermore, *in vitro* studies have demonstrated the potential of extracellular vesicles to transfer mRNA, miRNA and proteins to renal cells[210].

MSCs produce and secrete anti-apoptotic, mitogenic and proliferative growth factors, as well as factors that promote angiogenesis, such as VEGF, hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1), adrenomedullin and stromal cell-derived factor 1 (SDF1)[211–214]. These factors can be transferred by MSC-derived extracellular vesicles to tubular cells. For example, MSC-derived exosomes transfer IGF1 to tubular cells[215]. Extracellular vesicles from MSCs preconditioned with erythropoietin exhibited an enhanced renal protective effect[216]. Potentially, bone marrow dendritic exosomes could be used similarly, to potentiate or modulate T cell responses[106,217,218].

Importantly, not only bone marrow MSCs but also the microvesicles themselves can be injected as a treatment for AKI[79,216]. The microvesicles accumulate in the kidney[219], resulting in tubular cell proliferation and renal recovery by horizontal transfer of genetic material and modification of gene expression[144,220]. The beneficial effect on nephron recovery may also be related to uptake in endothelial cells and enhanced vascular

permeability[144,221]. Similar effects to those induced by MSCs were reported in rats injected with microvesicles from endothelial progenitor cells[222] and in mice injected with human liver stem cells or extracellular vesicles derived from them[223]. Clinical studies have, as yet, not investigated the capacity of MSC-derived extracellular vesicles to restore renal function in human AKI or chronic kidney disease.

***Chronic kidney disease and renal failure.*** Patients with uraemia have elevated levels of platelet-derived, neutrophil-derived, erythrocyte-derived and endothelial cell-derived extracellular vesicles[28,166,224–227]. Platelet-derived extracellular vesicle levels were elevated regardless of dialysis treatment (haemodialysis, continuous ambulatory peritoneal dialysis or no dialysis), which again suggests that dialysis does not remove the vesicles. On the contrary, the level of platelet-derived and neutrophil-derived microvesicles increased after haemodialysis sessions[166]. Patients who had thrombotic events had higher levels of platelet-derived extracellular vesicles[224] and the extracellular vesicle fraction of patient plasma induced thrombin generation in normal plasma[225], suggesting that the vesicles are prothrombotic. The presence of an arteriovenous fistula did not affect the level of extracellular vesicles but erythropoietin treatment possibly enhanced levels. Patients with uraemia also exhibit enhanced atherosclerosis, a process in which extracellular vesicles are explicitly involved (see below).

Baseline levels of endothelial cell-derived extracellular vesicles could predict cardiovascular outcome in uraemic patients[228]. Endothelial extracellular vesicle levels correlated with vascular dysfunction, as evaluated by loss of flow-mediated dilation, increased aortic pulse wave velocity and increased common carotid artery augmentation index[226], and inversely correlated with brachial artery and aortic shear stress[229]. *In vitro* studies using patient-derived extracellular vesicles showed decreased endothelial relaxation (inhibition of noradrenaline contraction); in particular, patient-derived endothelial extracellular vesicles decreased nitric oxide release. Importantly, the uraemic toxins indoxyl sulfate and p-cresol induced the release of extracellular vesicles from human umbilical vein endothelial cells (HUVECs) *in vitro*[28].

In addition to circulating extracellular vesicles, urinary exosomes have also been assayed in chronic kidney disease, and specific miRNAs, such as miR-29c, and the mRNA encoding CD2AP, correlated with kidney function and renal fibrosis[230,231], which could potentially be used as noninvasive markers of these disease features.

***Renal transplantation.*** The immunostimulatory or immunosuppressive and procoagulant potential of extracellular vesicles suggest that they could be used as biomarkers after renal transplantation, but may also contribute to post-transplantation complications. A prospective study investigated the levels of microvesicles derived from platelets, leukocytes in general, granulocytes in particular, and erythrocytes in renal transplant recipients showing a significant decrease in levels in the months after transplantation. Tissue factor activity in the microvesicle fraction decreased but remained elevated in comparison to controls. Microvesicle levels exhibited an inverse correlation to renal function. Furthermore, patients with cardiovascular disease (CVD) had less of a decrease in microvesicle levels post-transplantation than those without CVD[232]. Urinary CD133-positive microvesicles, derived from nephron cells, were shown to be biomarkers of delayed graft function and vascular damage[233].

Extracellular vesicles may be involved in acute rejection following renal transplantation. Ultramorphological studies of transplant biopsy samples showed that the antibody-mediated renal allograft rejection exhibited microvesicles in peritubular capillaries alongside platelet deposits and endothelial injury[234]. Antigen-presenting vesicles may activate an anti-donor T cell response, possibly by transfer of dendritic cell exosomes[218]. Similarly, cytomegalovirus (CMV)-infected endothelial cells activate $CD4^+$ T cells from CMV-positive individuals depending on the contaminating presence of class II MHC-expressing antigen-presenting cells within the T cell population. The CMV antigens are transferred to the antigen-presenting cells via exosomes *in vitro*. This mechanism may contribute to CMV-induced allograft rejection or chronic allograft vasculopathy[99].

Treatment of graft versus host disease or acute rejection with anti-thymocyte globulin (ATG) induces thrombocytopaenia and a coagulation disorder that is characterized by increased plasma levels of D-dimer and thrombin–antithrombin complexes. ATG binds to platelets and can be detected on platelet-derived microvesicles with complement activation[235]. Complement-induced platelet activation by ATG can thereby induce aggregation and release of microvesicles. Similarly, calcineurin inhibitors induce the release of endothelial cell-derived microvesicles capable of activating complement in plasma[236].

Interestingly, urine samples from renal transplant recipients with acute rejection, both T cell-mediated and antibody-mediated, showed a distinct mRNA profile that correlated with acute rejection, distinguishing the samples from those obtained from patients with acute tubular injury without rejection and also distinguishing T cell-mediated rejection from antibody-mediated rejection[237]. Moreover, certain long non-coding RNAs, possibly released within tubular microvesicles, have been detected in the urine of patients with acute rejection and correlated with renal function 1 year after transplantation[238].

Using proteomics on urinary exosomes from renal transplant recipients with tubular injury, cell-mediated rejection, or antibody-mediated rejection, followed by bioinformatic analysis, one study identified profiles of proteins associated with certain pathologies[239]. Specific markers were analysed in the exosomal fraction of urinary samples showing that neutrophil gelatinase-associated lipocalin (NGAL), produced in the distal nephron, could be used as a biomarker of delayed graft function ischaemia–reperfusion injury, and of longer cold ischaemia time in allografts from cadaver donors[240].

Both urinary exosomal NGAL and IL-18 correlated with decreased creatinine levels after transplantation[241]. Urinary exosomal aquaporin 1 was decreased in rats subject to ischaemia–reperfusion as a model of AKI, and also in a patient that received a renal transplant[242], which the researchers suggested could be used as a marker of ischemia–reperfusion injury.

## Diseases associated with renal dysfunction

The release of extracellular vesicles is associated with disease states such as malignancies[243], inflammatory diseases, thrombotic conditions, atherosclerosis, metabolic syndrome and hypertension, diabetes mellitus (see below), neurodegenerative disorders[244], sickle cell anaemia[245] and pre-eclampsia[246]. In these conditions, and others, extracellular vesicles bearing specific antigens can be diagnostic biomarkers of disease but they may also contribute to disease pathophysiology. We highlight the role of extracellular vesicles in several clinical conditions that lead to renal dysfunction.

***Inflammatory disorders.*** Extracellular vesicles can induce the release of cytokines and chemokines from cells and can transfer chemokine receptors to recipient cells — effects that contribute to inflammation[25,90,102]. Extracellular vesicles are elevated in the circulation during autoimmune disorders[247,248], and are particularly studied in systemic lupus erythematosus (SLE), in which levels of endothelial extracellular vesicles correlated with the disease activity score, glomerulonephritis, hypertension, history of arterial thrombosis, and lipidaemia[249]. Extracellular vesicles may also carry immunoglobulins and C1q[250] and contribute to immune deposits in glomeruli and to inflammatory activation[251]. Interestingly, levels of urinary exosomal miR-29 were inversely correlated with renal fibrosis in lupus nephritis, which could be utilized as a biomarker of disease progression[252]. In patients with rheumatoid arthritis, levels of platelet-derived microvesicles correlated with disease activity[253] and induced synovial fibroblasts to release cytokines within the joint cavity in a murine model[254].

***Thrombotic conditions.*** The procoagulant and prothrombotic effects of extracellular vesicles have been assessed in thrombotic conditions, such as venous thromboembolism, anti-phospholipid syndrome and paroxysmal nocturnal haemoglobinuria (PNH). In patients with venous thromboembolism, higher levels of extracellular vesicles were detected in pilot studies[255], mainly of endothelial-derived microvesicles and their aggregates with monocytes[256], which could possibly be used as biomarkers for detection of venous thromboembolism and to guide prognosis in combination with other markers[257]. Elevated levels of procoagulant extracellular vesicles were detected in patients with acute pulmonary embolism, and could possibly constitute a risk factor for this condition in conjunction with other cardiovascular risk factors[258].

Anti-phospholipid syndrome is an autoimmune condition that is associated with circulating anti-phospholipid antibodies and an increased risk of thrombosis and obstetric complications. Patients with anti-phospholipid syndrome were found to have elevated levels of circulating endothelial cell-derived extracellular vesicles, and plasma containing anti-phospholipid antibodies induced the release of procoagulant extracellular vesicles from endothelial cells[259], suggesting that endothelial cell-derived extracellular vesicles may contribute to the disease phenotype[260].

PNH is a rare disease caused by mutations in the phosphatidylinositol glycan class A (*PIG-A*) gene, with deficiency of glycosylphosphatidylinositol (GPI)-linked proteins including the cell-bound complement regulators CD55 and CD59 (REF. 261). Manifestations of this disease include haemolytic anaemia, thrombosis, renal manifestations or renal failure. Patients with PNH were shown to have elevated levels of endothelial cell-derived and platelet-derived extracellular vesicles in their circulation[262], which could contribute to thrombotic complications.

***Atherosclerosis, diabetes mellitus and metabolic syndrome.*** Extracellular vesicles derived from multiple cells accumulate in the lipid core of atherosclerotic plaques[263]. In the context of atherosclerosis, the potential of extracellular vesicles to communicate between cells, and their distinct proinflammatory and thrombogenic characteristics, have been shown to contribute to the disease process in multiple studies (reviewed elsewhere[264–266]) and are therefore not discussed in this Review.

Increased levels of circulating extracellular vesicles have been found in patients with type 1 or type 2 diabetes mellitus[267] but with differences in the cells they are derived from. Correlations were found between certain subpopulations of extracellular vesicles and diabetic complications, such as diabetic nephropathy[268] and coronary artery lesions[269]. Type 1 diabetes mellitus was associated with elevated levels of platelet-derived and endothelial cell-derived extracellular vesicles and the latter were associated with albuminuria[270]. Patients with type 1 or type 2 diabetes mellitus exhibited elevated levels of phosphatidylserine exposing-annexin V-positive blood cell-derived extracellular vesicles that were associated with procoagulant activity and HbA1c levels. Patients with type 2 diabetes mellitus had circulating tissue factor-positive extracellular vesicles originating from T lymphocytes, granulocytes and platelets[271], and a correlation was found between circulating extracellular vesicles and arterial stiffness[272]. The content of urinary extracellular vesicles may be used in future studies to monitor the progression of diabetic nephropathy, as has been suggested by one pilot study that investigated a reduction in exosomal regucalcin[273]. Islet cells prepared for transplantation were shown to release tissue factor-positive extracellular vesicles, associated with insulin and glucagon granules, and these vesicles had a negative effect on the outcome of transplantation[274].

Patients with metabolic syndrome and cardiovascular risk factors were shown to have elevated levels of platelet-derived extracellular vesicles[275]. Weight reduction in individuals with obesity decreased the level of platelet-derived extracellular vesicles[276], which corroborates *in vivo* data

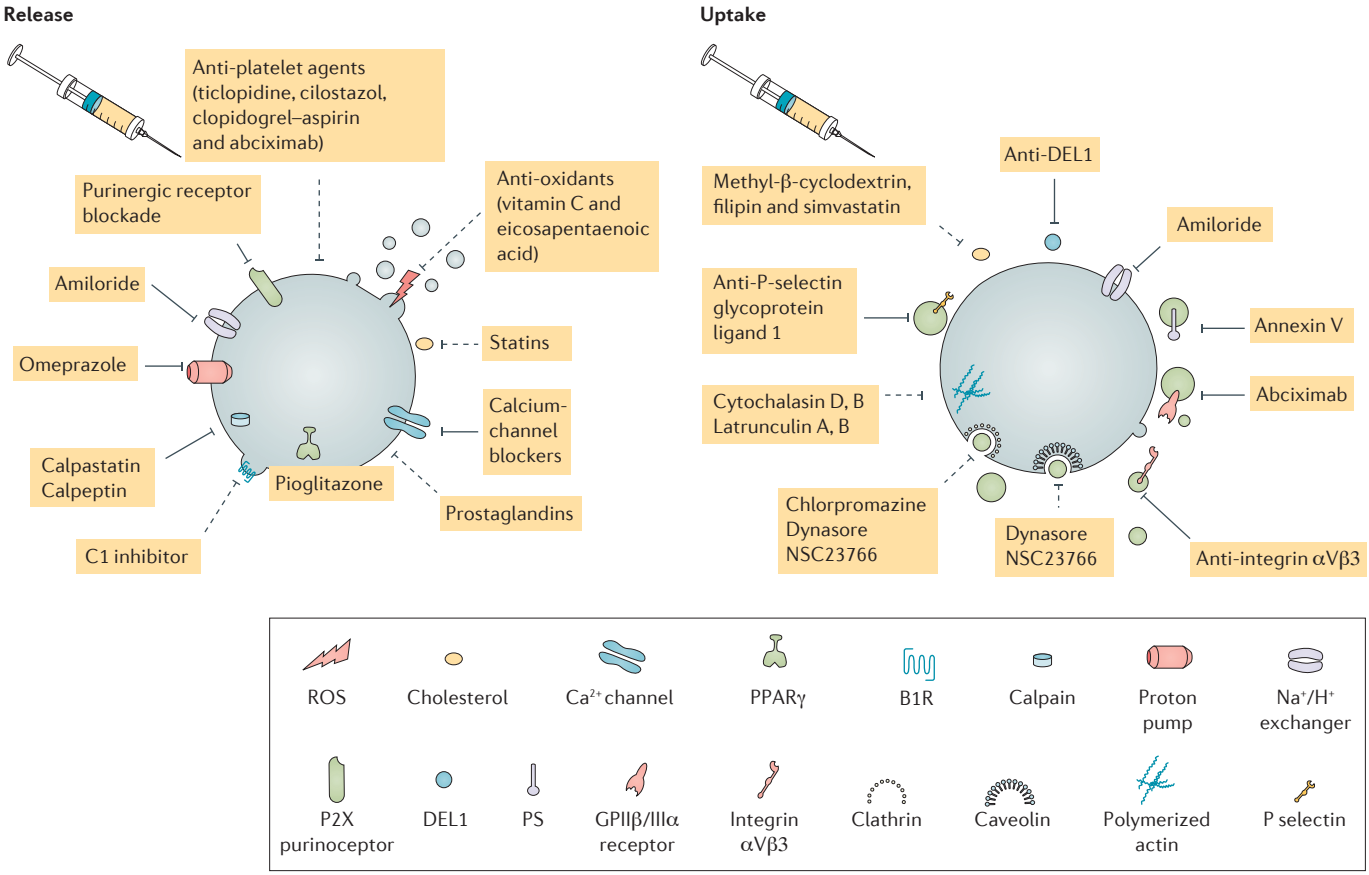


Figure 4 | **Drugs that block extracellular vesicle release and uptake.** Several substances can block the release or uptake of extracellular vesicles. A dashed inhibitory line indicates that the process involved in the release or uptake of extracellular vesicles is blocked by the indicated substance. A solid inhibitory line indicates that a specific receptor or protein on the cell membrane or within the cells is blocked by the indicated substance. B1R, kinin B1 receptor; DEL1, developmental endothelial locus 1; GPIIb/IIIa, glycoprotein IIb/IIIa; PPAR, peroxisome proliferator-activated receptor; PS, phosphatidylserine; ROS, reactive oxygen species.

showing that an increased intake of fat induced excessive levels of proinflammatory extracellular vesicles[277]. These results were, however, challenged in individuals with severe obesity in which weight reduction did not decrease the level of extracellular vesicles[278]. The level of endothelial cell-derived extracellular vesicles was also elevated in patients with metabolic syndrome and these were found to impair nitric oxide-dependent endothelial relaxation *in vitro* and *in vivo*[279]. Certain subpopulations of endothelial cell-derived extracellular vesicles were specifically increased, exhibiting PECAM-1 and enhanced binding to leukocytes[280]. Proteomics analysis of the content of plasma microvesicles in patients with CVD showed that the proteins were mainly involved in biological functions such as platelet activation, coagulation and adhesion[281], and circulating miRNAs presented a risk-associated profile associated with the metabolic syndrome[282].

***Hypertension.*** Patients with severe, untreated hypertension had higher levels of platelet-derived and endothelial cell-derived extracellular vesicles compared with patients with mild hypertension or non-hypertensive controls[283]. Endothelial extracellular vesicles levels, as a biomarker of impaired endothelial function, indicated that vascular injury persisted even when blood pressure was under control[284]. In addition, extracellular vesicles from endothelial cells (possibly apoptotic bodies) may be elevated in patients with microalbuminuria[285]. A protein profile of extracellular vesicles in urine identified certain proteins specifically associated with hypertension[61]. In urine of patients with renovascular hypertension and lower estimated glomerular filtration rate (eGFR), elevated podocyte-derived extracellular vesicles were detected in comparison to patients with essential hypertension and preserved eGFR[286].

## Extracellular vesicles and therapy

***Extracellular vesicles as delivery systems for therapeutics.*** The capacity of extracellular vesicles to shuttle proteins and nucleic acids from one cell to another may be utilized for therapeutic purposes, particularly as certain extracellular vesicles preferentially bind to specific cells and could thus deliver a drug, a ligand mediating a receptor-induced signal, an altered protein or an RNA, to affect cellular processes[24]. For example, plasma-derived exosomes can be loaded with

exogenous small interfering RNA (siRNA) that can be delivered to blood cells[287]. The homing of extracellular vesicles to specific target cells can be achieved by genetically engineering the parent cell from which the extracellular vesicles are released so that a specific ligand is expressed[288]. In addition, extracellular vesicles can cross barriers such as the blood–brain barrier[289] and the glomerular basement membrane[14], and thus reach protected regions. A limited number of studies have reported that exosomes can be loaded with chemotherapeutic agents that can be delivered to malignant cells[290], a topic that has been reviewed elsewhere[35,291]. Phase I and phase II clinical trials have shown that administration of dendritic exosomes to patients with malignancies is feasible[292] and this is a promising area of research. A position paper on the subject of extracellular vesicles as therapeutics has been published by the International Society for Extracellular Vesicles[293].

***Extracellular vesicles as potential therapeutic targets.*** Extracellular vesicles are continuously being cleared from the circulation. The half-life of platelet-derived microvesicles could be as short as 10 minutes[294]. During their rather short half-life, extracellular vesicles exhibit potent effects that may be harmful to the host. Therefore, treatments that decrease extracellular vesicle release or uptake, at least temporarily, may be beneficial during sepsis, inflammation or thrombotic disease (FIG. 4). A variety of pharmacological agents have been found to decrease the level of blood cell-derived and endothelial cell-derived microvesicles in patients, as has been reviewed elsewhere[295]. These agents include antiplatelet agents, antioxidants, statins, calcium-channel blockers and prostaglandins[296–299]. Pioglitazone, a selective ligand of peroxisome proliferator-activated receptor γ, reduces the level of endothelial cell-derived microvesicles in metabolic syndrome[300].

Our group has shown that incubation of plasma with C1 inhibitor reduces the release of chemotactic kinin B1-receptor-positive endothelial microvesicles, which may have potential for the treatment of inflammatory diseases, such as vasculitis[57]. The calpain inhibitor calpeptin was reported to decrease the shedding of platelet-derived microvesicles *in vitro*[301]. Calpastatin, which also inhibits calpain, reduced the shedding of microvesicles in a mouse model of sepsis[302]. Proton pump inhibitors, such as omeprazole, decrease the release of exosomes from tumour cells[303]. Similarly, amiloride decreases exosome production[304] and microvesicle cellular uptake[305]. Stimulation of purinergic receptors induces shedding of microvesicles[29–31] and our group has shown that blocking P2X receptors decreased the shedding of microvesicles from red blood cells[56]. Furthermore, extracellular vesicles could potentially be removed from plasma by immunoadsorption using specific antibodies or ligands[206].

In addition to reducing the level of microvesicles, certain substances and antibodies may decrease their uptake by cells, as reviewed elsewhere[54]. The uptake of platelet-derived microvesicles by endothelial cells was decreased by an antibody to DEL1, by annexin V, by abciximab (an antagonist of glycoprotein IIb/IIIa), by anti-integrin αVβ3 and by chlorpromazine[54,78]. Cytochalasin D, which depolymerizes actin and interferes with the formation of microfilaments and macropinocytosis, also blocked the uptake of platelet-derived microvesicles by endothelial cells[305]. Similarly, cytochalasin B decreased reticulocyte-derived exosome uptake by macrophages[306]. The uptake of monocyte-derived microvesicles by platelets was blocked by annexin V and by an antibody to PSGL-1 (REF. 10). Although reduction of cellular uptake will increase the levels of circulating extracellular vesicles, it will reduce the effects associated with vesicle interactions with recipient cells.

## Conclusions

Extracellular vesicles participate in intercellular communication in physiological as well as pathological processes. Their excess release during inflammatory and thrombotic disorders may be harmful, whereas microvesicles derived from MSCs can be beneficial in renal repair. Microvesicles rid cells of unwanted substances and thus pharmacological interference with the shedding of microvesicles may be potentially damaging to cells, but temporary decreases may theoretically be favourable in renal diseases in which microvesicles promote infection and/or renal failure. Future studies will extend our knowledge on the role of extracellular vesicles in disease processes, as novel therapeutics and as targets for the treatment of renal disease.


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All authors contributed to researching data for the article, discussion of the content, and revising or editing the manuscript before submission.

**Competing interests statement**
The authors declare no competing interests.



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