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Nanomedicines for renal disease: current status and future applications

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Abstract

Treatment and management of kidney disease currently presents an enormous global burden, and the application of nanotechnology principles to renal disease therapy, although still at an early stage, has profound transformative potential. The increasing translation of nanomedicines to the clinic, alongside research efforts in tissue regeneration and organ-on-a-chip investigations, are likely to provide novel solutions to treat kidney diseases. Our understanding of renal anatomy and of how the biological and physicochemical properties of nanomedicines (the combination of a nanocarrier and a drug) influence their interactions with renal tissues has improved dramatically. Tailoring of nanomedicines in terms of kidney retention and binding to key membranes and cell populations associated with renal diseases is now possible and greatly enhances their localization, tolerability, and efficacy. This Review outlines nanomedicine characteristics central to improved targeting of renal cells and highlights the prospects, challenges, and opportunities of nanotechnology-mediated therapies for renal diseases.

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Author contributions

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

Competing interests statement

O.C.F. has financial interests in Selecta Biosciences, Tarveda Therapeutics, and Placon Therapeutics. D.A.A serves on the Board of Directors of Pfizer, Alnylam Pharmaceuticals, Seres Therapeutics, Tarveda Therapeutics and Placon Therapeutics. All other authors declare no conflicts.

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Nanotechnology involves the engineering of atoms and molecules at the submicron scale. The unique optical, electrical, physical, and chemical properties of matter at this scale can yield materials that behave differently from their macroscale counterparts. Inorganic or organic multifunctional nanocarriers, which we refer to as nanoparticles (NPs), can be engineered to deliver drugs or imaging agents with unique characteristics. For example, NP delivery improves pharmacokinetics and biodistribution and enables targeted delivery of drugs to specific tissues, cells, or subcellular compartments. Additionally, achieving spatiotemporal, triggered, and controlled release of a variety of payloads (such as small-molecule drugs, contrast agents, peptides, proteins, deoxy and ribonucleic acids, and their combinations) over extended periods might be possible. Nanomedicines are typically degradable, biodegradable, and bioeliminable structures generally <150 nm in size that can incorporate the aforementioned payloads[1–5]. Physicochemical properties of nanomedicines such as composition, size, geometry and/or shape, surface charge, surface chemistry (including targeting ligands), hydrophobicity, roughness, rigidity, and elasticity influence their uptake and/or ability to target particular organs and cells[6–13] (FIG. 1).

In the 1970s, colloid-based drug delivery agents designed to improve drug therapeutic efficacy and reduce toxicity were termed NPs[14]. The term ‘nanoparticle’ is mentioned in over 135,000 publications, and the number of publications on this topic has doubled every other year between 2000 and 2014 (PubMed). Though this rapid growth seems to be slowing, the absolute number of articles published on the topic remains impressive at ~15,000 per year. Monoclonal antibodies took decades to gain significant momentum; similarly, the broad impact of nanotechnology-based therapeutics will become more apparent as these technologies mature[15]. Exciting developments in NP design, such as the generation of building blocks that release drugs in response to stimuli from the disease environment (such as increased enzymatic activity, the presence of reactive oxygen species or lowered pH) or to externally delivered stimuli (such as heat or ultrasound), are laying the foundations for ‘prompted’ and ‘modular’ drug delivery[16–23]. Additionally, the ability to co-deliver synergistic therapeutics will further enlarge the arsenal of nanomedicines for the treatment of a variety of diseases and facilitate important combination therapies[24,25].

Nanomedicine-based genetic therapies directed to the liver, an organ that filters significant volumes of blood, have now been successfully developed[26,27]. Such established design principles can also be used to create NPs that can bind to, be taken up by and retained by diseased renal cells. Numerous *in vivo* NP studies initially designed to target tumours have resulted in serendipitous discoveries of principles underlying kidney targeting and selective accumulation. Such findings raised important ‘structure–activity’ correlations, which can be used to influence NP retention and clearance from the general circulation within kidneys, and these insights could be applied to overcome challenges in the generation of kidney targeted nanomedicines.

In this Review, we discuss the application of nanotechnology to diseases of the kidney. We introduce fundamental concepts of nanomedicine, examine renal anatomy and selective permeability in health and disease, and highlight nanotechnology research aimed at targeting the major renal membranes and cell types involved in disease. Furthermore, we discuss microfluidic- based kidney models and artificial organ-on-a-chip approaches to investigate

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synthesis, screening and testing of NPs for renal disease therapy. Finally, we examine challenges in the translation of nanomedicines to the clinic.

Nanomedicines

Structure

Depending on the method of preparation or the chemical nature of the building blocks used, NPs can have either a solid or hollow aqueous core (FIG. 1a). Their surface layer can be coated with inert polymers that can shield the NP from blood components. To date, polyethers such as polyethylene glycol (PEG) have been mostly used for this purpose. A variety of payloads can be incorporated within the core or surface layers of the NPs: hydrophilic or hydrophobic small-molecule drugs, proteins, peptides, nucleic acids, or imaging agents. The surface of the NPs can be functionalized with ligands targeted to specific receptors or proteins of interest to achieve ‘active targeting’. Targeting ligands can be antibodies, antibody fragments, antibody mimetics, proteins, peptides, nucleic acids (such as aptamers) and small-molecule ligands (FIG. 1b). Physical characteristics such as NP size can also be used to achieve ‘passive targeting’ and enhanced accumulation in tumours (FIG. 1b). Liposomes were the first nanomedicines to enter the clinic and be marketed, and therefore represent a large portion of clinical- stage nanomedicines. They were followed by polymer–drug conjugates, polymeric micelles, polymeric NPs, protein-based NPs and dendrimers (TABLE 1)[15,28].

Current therapeutic uses

The majority of nanomedicines generated so far have been developed for cancer therapy, as compromised endothelial barriers in tumours facilitate NP retention (discussed below). Over a dozen NP platforms based on liposomes[29–45], albumin[46–51], polymeric micelles[52–58], and nanosized polymer-drug conjugates[59–62] have been approved by the FDA (TABLE 1). A few targeted NPs including Her2 scFv-targeted liposomes (MM-302)[63], the first targeted controlled-release polymeric NP BIND-014 (REF. 64), and the first targeted short interfering (si)RNA NP CALAA-01 (REF. 65) are currently being tested in clinical trials for cancer therapy. Although many nanomedicines improve the pharmacokinetics and biodistribution of drugs, so far, only one nanomedicine, CPX351, has increased survival in patients with cancer when directly compared with the conventional parent drug[66]. These findings underscore the need to rethink strategies for the development of NPs, including potential patient selection to identify those most likely to respond to nanomedicines.

Nanotechnology-enabled diagnostic and therapeutics based on dendrimers[67–69], gold[70–72], silica[73–75], iron oxide[71,76,77] and hafnium oxide[78,79] are also currently under clinical investigation, with the majority intended for oncological applications (TABLE 1). For example gold NPs decorated with a surface of PEG conjugated to TNFα molecules are in phase I/II trials for solid tumour therapy[80]. Silica NPs in combination with gold are being tested in thermal ablation therapy of head and neck cancers[81]. Two MRI contrast agent iron-oxide NPs (Ferumoxtran-10® and Ferumoxytol®) are in clinical trials for cancer imaging[82,83]. A therapeutic version of iron oxide NPs termed NanoTherm® was approved in Europe in 2010 for the thermal ablation of glioblastomas (magnetic hyperthermia

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treatment)[84]. Clinical trials have begun for the investigation of hafnium oxide NPs (phase I), which will be used as radiosensitizers in patients with soft-tissue sarcomas[78]. Nanomedicines are also undergoing clinical translation for gene therapy[85], RNA interference[26,65,86–88], and immunotherapy[89,90]. Viral NPs have found utility in the delivery of a range of therapeutics and have been clinically validated for gene therapy applications[91,92,93]. For example, the tumour-homing oncolytic pox virus was engineered to generate JX-594, a particle that expresses granulocyte colony-stimulating factor (G-CSF) to potentially increase the immunological antitumour response[94]. Exosomes are being explored as targeted vectors in drug delivery, where their signalling properties can be exploited to manipulate cellular proteomic functions beyond their current applications in kidney disease biomarker discovery[95–98]. Other novel nanomaterials including nanodiamonds[99–101] and nanographene[102,103] are receiving attention in drug- delivery applications: the adsorption of hydrophobic drugs to these surfaces facilitates the delivery of poorly soluble therapeutics in high concentrations.

Developments in nanomedicine have facilitated investigations into the delivery of multiple synergistic active pharmaceutical ingredients[25,104,105]. For example, the liposomal formulation of cytarabine and dauno-rubicin CPX-351 is under development for high-risk acute myeloid leukemia[66]. Design advances have also produced nanomedicines that can simultaneously deliver diagnostic and therapeutic agents, termed theranostics[106]. For example, liposomes containing an MRI contrast agent and an anticancer siRNA payload can be used to dynamically monitor and cause tumour regression[107]. Nanotheranostic agents that use near infrared absorbing agents to convert optical energy into heat for ablating tumour cells have also been developed and are used for image-guided photothermal therapy with inorganic NPs to treat tumour metastases[108–110].

The versatility of NPs (antigens, adjuvants and targeting moieties can be incorporated into single NPs) has also led to their utilization as synthetic vaccines[111,112]. NPs can accumulate in lymph nodes, target antigen- presenting cells, and co-deliver antigens and/or adjuvants that can trigger impressive cellular and/or humoral responses[111–117]. The co-delivery of an antigen with a TLR7, TLR8 or TLR9 agonist in polymeric NPs strongly increased humoral and cellular immune responses with minimal systemic production of inflammatory cytokines in mice[111,112]. This strategy enables the use of TLR agonists as vaccine adjuvants for indications where cellular immunity or a robust humoral response is required.

To date, the vast majority of renal drug delivery studies have mostly investigated the applications of nanosized polymer–drug conjugates, liposomes[118–131], or inorganic nanodiagnostic compounds to study renal toxicity[132–140]. Although organ biodistribution profiles of numerous NPs have been evaluated for other indications than renal diseases (such as tumour therapy), they are quite informative regarding NP structure–activity relationships (concerning NP size and charge) in the kidneys. In glomerular nephropathies, NPs accumulate in the glomeruli, perhaps due to increased vascular permeability and inflammation[127,130,141,142]. Despite these findings, the kidney has largely been overlooked as a target organ for NP-mediated drug delivery. Few systematic studies of NP glomerular deposition in various kidney diseases and disease stages have been conducted with

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nanomedicines; however, the ‘tuneable’ properties of NPs and their inherent tendency for glomerular deposition might be exploited for the treatment of kidney diseases.

NPs and systemic circulation

Nanomedicines are currently typically administered via intravenous injection, although research on their oral delivery is under way (with the promise of greater patient compliance)[143–147]. The biological and physicochemical properties of NPs (FIG. 1b) influence their interactions with biological surroundings and affect their *in vivo* circulation and biodistribution[148,149].

The protein corona

Blood contains ~3,700 distinct proteins, and a few tens of them can adsorb to NP surfaces, forming a ‘protein corona’ (REFS 10,150–156). Adsorption of some plasma proteins (such as immunoglobulins, complement proteins, and fibrinogen) can facilitate clearance by the mononuclear phagocyte system[157] and limit NP circulation; conversely, binding of dysopsonin proteins (such as apolipoproteins and albumin) might prolong NP presence in the systemic circulation[154–156]. Thus, the impetus towards understanding NP interactions with their surroundings *in vivo* is strong as the protein corona can bestow a new biological identity to NPs[158,159].

Surface modifications

To increase NP circulation time, PEGylation of the NP surface to minimize protein binding and render it hydrophilic has been routine practice since the mid-1970s[160]. Doxil, the first FDA-approved PEGylated ‘stealth’ liposome formulation, has a circulation half-life of up to ~2 days[45]. Further investigation of the function of the protein corona, however, yielded contradictory results. For example, a 2016 study showed that PEGylated polystyrene NPs are highly prone to selective adsorption of clusterin (a 80 kDa chaperone protein), which reduced their nonspecific macrophage uptake *in vitro*[161]. These findings and other results suggest that the stealth effects of PEGylation could be mediated by changes in the nature of the protein corona[161,162]. Formation of the protein corona can also reduce the efficacy of the targeting ligands coupled to NPs[162] or, in some instances, promote organ-specific accumulation, as in the case of apolipoprotein-E-mediated targeting of NPs to hepatocytes *in vivo*[163]. Predicting such *in vivo* responses will require careful analytical techniques and protein fingerprinting to better understand how the NP corona influences the pharmacokinetics, biodistribution, intrarenal biodistribution, and therapeutic efficacy of NPs[164–167]. In addition, a few animal and human studies have shown potential PEG immunogenicity (presence of anti-PEG antibodies) leading to rapid clearance of PEGylated NPs after repeated administrations[168–171]. The majority of these studies are based on liposomal NPs and further studies are needed to ascertain the exact immunogenic mechanisms associated with PEGylated NPs.

Pharmacokinetics

Numerous studies in tumour therapy show that NPs can enhance drug delivery, and improve drug C_{max}, pharmacokinetics and plasma area under the curve (AUC; a measure of total

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drug exposure over time), compared to the standard dose and formulation[172–175]. Hence, NP versus drug pharmacokinetics; encapsulated versus free circulating drugs; drug versus NP C_{max}; drug versus NP AUC; and parameters that can further affect plasma versus kidney pharmacokinetics and AUC are important factors to consider for the development of effective nanomedicines for the kidney. The infusion time of drugs that are administered intravenously can vary and drugs usually reach their plasma C_{max} during the period of infusion, after which the concentration of the drug diminishes[176,177]. With NP-encapsulated drugs, the plasma concentration of the released drug is initially low and increases progressively to reach its C_{max} at a slower rate than that of the conventional drug[64]. Of note, the C_{max} after drug release from NPs rarely reaches the levels achieved with intravenous injection of the free drug. This kinetic is a major advantage of nanomedicines, as it could reduce drug-associated toxicity, which depends on C_{max}[64]. The free drug and the NP-released drug have similar plasma AUCs, although that of the released drug is generally broad and flat, whereas that of the free drug peaks and has a tail[64]. These findings imply that NPs might reduce C_{max}-associated toxicity, but not AUC-related toxicity, as the total dose of the drug is still released by NPs, albeit at a reduced rate.

Renal anatomy and selective permeability

The adult human kidney can contain 1–2.5 million nephrons, which maintain fluid homeostasis, osmoregulation, and waste filtration[178]. A single nephron consists of two filtration units: the glomerulus and a hairpin-shaped tubule composed of a proximal tubule, the loop of Henle, a distal tubule, and collecting duct[179]. The glomerulus comprises glomerular endothelial cells (GECs)[180], a glomerular basement membrane (GBM)[181], podocytes[182], mesangial cells[183], and parietal epithelial cells[184] (FIG. 2).

Three distinct glomerular filtration barriers separate the vasculature from the urinary spaces and selectively filter blood molecules and ions, yielding a primary urinary filtrate: GECs, the GBM and podocytes[179]. In the nephrons, the renal tubules and collecting ducts concentrate and balance the urinary filtrate via resorption and secretion of ions, water and certain nutrients through channels and transporters[185]. Under normal conditions, blood filtration by size and charge by the glomerular barrier ensures that only water and small solutes (urea, glucose, amino acids, and mineral ions) from the plasma cross to the urine[179]. High molecular-weight plasma components, such as erythrocytes, and negatively charged components, such as albumin, are retained in the blood[179]. Electrostatic repulsion is thus a predominant filtration parameter, as the glomerular filtration barrier strongly favours the passage of anionic molecules, as shown by the glomerular permeability to larger neutrally charged dextran polymers[186]. The impairment of this barrier leads to proteinuria, a hallmark of glomerular diseases[187].

To escape the general circulation, a NP must cross the glomerular filtration barrier (FIG. 2). Structural features of the glomerular barrier in the setting of renal disease, in particular enlarged endothelial gaps (detailed below), can be exploited for NP delivery to various kidney cells and components.

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NP size

NP size has been widely explored to design effective nanomedicines. Most therapeutic NPs are 30–150 nm and are not subject to kidney filtration into the urine, unless they are degraded into particles <10 nm, or the glomerular filtration barrier is damaged by disease (FIG. 2).

In healthy states, colloids and particles with a hydrodynamic diameter up to 5–7 nm fall below the kidney filtration threshold, pass through the glomerulus and are excreted[188]. NPs below 5.5 nm can be excreted via renal clearance, with an efficiency of >50% of the injected dose 4 h after administration[188,189]. Of note, some deviations from this threshold have been observed for 12–16 nm inorganic NPs, suggesting that case-by-case investigation of the renal clearance of small-sized NPs is important, as each type of NP has a distinct chemical composition and size-distribution profile[133].

Kidney accumulation studies based on size have shown that NP accumulation is restricted to the glomerular mesangium (driven by mesangial cell uptake) and the kidney extracellular matrix, with maximal glomerular deposition for ~80 nm NPs[190]. One caveat of these studies is that most have been conducted in healthy animals, in which the renal filtration barriers are intact. Considering the influence of NP size on the biodistribution and clearance of nanomedicines, and on size-dependent renal transport, is therefore a crucial parameter for renal nanomedicines.

NP shape

The transport of NPs in the circulation is influenced to a greater degree by the applied convective forces in blood than by Brownian motion. Therefore, shape has an important effect on *in vivo* performance and biodistribution of NPs, which exist in a wide range of geometries[191]. For example, a top-down fabrication method termed particle replication in non-wetting templates (PRINT) utilizes lithography techniques to create polymeric NPs of a wide variety of geometries, shapes, and aspect ratios[192,193]. Cylindrical and discoidal shapes are uniquely subject to blood flow (they have high aspect ratios and minimal regions of curvature), which affects their interaction with macrophages and cell membranes as particles with reduced curvature undergo faster internalization upon incubation with macrophages[194,195]. Similarly, nanoworm or nanorod (elongated cylindrical) structures show greater tumour accumulation than other shapes[196,197]. Non-spherical NPs marginate to vessel walls more efficiently than spherical NPs, although they are also more rapidly cleared depending on their aspect ratio and dimension[195,198,199]. In general, shapes that can accommodate cellular membrane wrapping processes are optimal for cellular internalization[200,201].

Interestingly, the kidney can also filter rigid NPs with high aspect ratios but with diameters smaller than the kidney filtration threshold, such as single-walled carbon nanotubes (SWCNTs) with a rod length of 100–1000 nm and diameter of 0.8–1.2 nm[199]. Even though SWCNTs have higher molecular weights (300–500 kDa) than plasma proteins (30–50 kDa), they are cleared by the kidney, suggesting that aspect ratio influences directional diffusion[199]. Similar NPs successfully delivered therapeutic siRNA to proximal tubule cells;

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fibrillar carbon nanotubes delivered two synergistic siRNA payloads targeting *Mep1b* and *Trp53*, which reduced the expression of key proteins involved in cisplatin-induced acute kidney injury[202]. One mechanism proposed to underlie this phenomenon is that hydrostatic forces orient carbon nanotubes perpendicularly to the GBM, enabling the insertion of their <10 nm axis. This shape might be optimal for targeting podocytes, which are difficult to access as they are protected by the fenestrated endothelial cells and the GBM[199,203] (FIG. 2).

NP charge

Highly positively charged NPs are rapidly cleared from the circulation by cells of the mononuclear phagocyte system owing to increased protein binding or high affinity interactions with phagocytic cells[6,204]. Negatively charged NPs can also be more subject to selective cellular uptake (by macrophages, for example) than NPs with neutral surfaces[205]. NPs with a surface charge of <15 mV had minimal macrophage uptake and long circulation times[206]. Anionic NPs (surface charge of −10.6 mV) had lower levels of liver and spleen accumulation than near-neutral polymeric micelles (1.3mV)[207]. Cationically charged NPs undergo high non-specific uptake in a variety of cells (cancer cells and macrophages), and can also achieve endosomal release[194]. This property is particularly important for the effective delivery of payloads for RNA interference (RNAi), which requires cytosolic delivery for efficacy[10,208–210].

Considerations regarding NP charge should also include the charge contribution of targeting ligands that are grafted on NP surfaces for retention in target tissue and/or receptor-mediated endocytosis, leading to increased intracellular drug concentrations[1,12,15,211] (FIG. 1). Charge selectivity is also an important criterion for kidney filtration. The suborgan distribution of intravenously administered ultrasmall anionic NPs (3.7 nm quantum dots) at the level of kidney cells was investigated using fluorescence imaging[212]. The majority of NPs were initially distributed within the peritubular capillaries or the glomerular arterioles, passed through the fenestrated glomerular endothelium, and were gradually taken up by the mesangial cells for up to 30 days[212]. Interestingly, only trace amounts of quantum dots could be detected in the urine. As the negative charge of the glomerular basement membrane prevents the filtration of anionic quantum dots, cationically charged quantum dots of similar size were found in much higher concentrations in the urine[212]. These studies provide a framework for understanding charge principles across kidney filtration barriers, which are predominantly negatively charged, in particular as applied to the ultrasmall inorganic NPs often used in imaging applications.

Targeting renal cells

The glomerular endothelial cell barrier

Blood capillaries in the kidneys are lined with GECs, which form the first part of the glomerular filtration barrier. They are characterized by fenestrations and transcellular holes (60–80 nm) filled with an endothelial glycocalyx. The glycocalyx (composed of podocalyxin, sialoglycoprotein, and podoplanin)[123] is a polysaccharide-rich >400 nm layer present on 30–40% of GEC surfaces[180,213]. This layer mechanically limits the access of

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circulating plasma components to endothelial cell membranes via a filamentous structure and strong negative charge[214]. The proteoglycan structure effectively makes the fenestrae narrow and restrictive, preventing passage of albumin from plasma, but facilitating water permeability[215].

The GECs and endothelial surface layer are important components of the glomerular filtration barrier. Impairment of the endothelial surface layer leads to albuminuria and vascular disease[123], and damage to this layer can lead to scarring and glomerulosclerosis[123]. Preclinical and clinical studies have shown that damage to the endothelial surface layer strongly correlates with diabetes-mellitus-induced proteinuria[216,217].

Lipidoid siRNA NPs effectively silenced multiple endothelial genes in the heart, lung, and kidney *in vivo* with minimal effects on gene expression in hepatocytes or immune cells[218]. Similar strategies can be used to deliver specific antisense[219,220], siRNA[26,65,86,87], mRNA[88], or DNA inhibitor oligonucleotides[85] to damaged GECs for repair and restoration of function. In a mouse model of glomerulonephritis, liposomes coated with an antibody directed against E-selectin, which is specifically expressed on activated endothelial cells during inflammation, were used to successfully deliver dexamethasone to $CD31^+$ GECs, which reduced glomerular endothelial activation and albuminuria after 7 days[221]. Such studies demonstrate the potential of using disease specific epitopes on the surface of GECs for targeted drug delivery. This approach is particularly beneficial as standard anti-glomerulonephritis therapies consist of glucocorticoids and cytotoxic drugs with systemic adverse effects.

The glomerular basement membrane

The GBM is an anatomic barrier of connective tissue composed of collagen IV, laminin, and proteoglycans (mostly agrin); these proteins are secreted by the GECs and podocytes[222]. The GBM is an integral component of the glomerular filtration barrier and acts as an intermediary sieving matrix, but can also accumulate pro-angiogenic ligands and secreted factors that mediate cellular communication between podocytes and GECs. Agrin, a major component of the heparin sulfate proteoglycan, has a high negative charge owing to core proteins such as syndecan, glypican, or biglycan with sulfated glycosaminoglycan side chains, which contribute to the net negative charge of the GBM[180,181]. The charge difference between the GBM and the blood has been proposed to be the main reason for the repulsion of plasma albumin (68 kDa, ~3.6 nm, negative charge).

Scanning electron microscopy studies showed that the GBM is a highly organized non-amorphous mesh-work of interconnected polygonal fibrils 4–10 nm thick[223]. In healthy states, the core of the GBM is more densely packed (10-nm pores) than the peripheral fibril network; however pores were enlarged up to 40 nm, in a rat proteinuric nephritis model[223] (FIG. 2). Subdiffraction resolution stochastic optical reconstruction microscopy (STORM) and STORM-electron microscopy showed that GBM-associated proteins have a highly oriented macromolecular organization of agrin, laminin, and collagen IV[224]. Mutations in the gene that encodes collagen IV disrupt the self-polymerized collagen network and cause Alport syndrome, a hereditary disease that stems from GBM dysfunction[225]. Mutation of the *LAMB2* gene, which encodes laminin subunit β2, prevents the assembly of the laminin

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complex LM-521 (a heterodimer composed of the laminin subunits β2, α5 and γ1), which leads to abnormal renal and neuromuscular phenotypes (Pierson syndrome), including splitting of the GBM[226]. Breakdown of the GBM in Pierson syndrome might be due to reduced levels of laminin trimer polymerization, a process that maintains basement membrane integrity[181].

The design of mRNAs targeted to either podocytes or GECs, both of which synthesize the matrix to form and maintain the normal composition of the membrane, might be necessary to repair mutations of the matrix proteins. For example, NPs bearing specific peptide sequences that bind to collagen IV target subendothelial collagen IV in the vasculature[227]. Interestingly, during renal clearance, cyclodextrin-containing polymer-based cationic NPs that contain siRNAs accumulate and disassemble at the GBM, which might have broader repercussions for NPs that assemble via electrostatic interactions between their matrix and payload[228]. Whether this property could be exploited to target the GBM is not yet clear. Using targeted NPs to silence GBM protein expression in order to reverse the effects of mutations in the genes encoding laminin and collagen IV would be an interesting avenue to explore in the treatment of hereditary diseases. In addition, NPs targeted to the GBM could help deliver drugs, mRNAs or siRNAs to podocytes and glomerular endothelial cells, because both types of cells sit on the GBM.

Podocytes

Podocytes have unique features such as foot processes and the slit diaphragm, a 40 × 140 Å-wide[229] bridge, mainly composed of nephrin, that connects juxtaposed foot processes. The slit diaphragm is porous but prevents the passage of large macromolecules such as proteins[230]. Since the discovery of nephrin (encoded by *NPHS1*) and podocin (encoded by *NPHS2*) as integral components of the slit diaphragm, several other podocyte-related proteins, such as glycogen synthase kinase 3b, have been discovered. Mutations in these proteins are linked to genetic diseases of the glomerulus[216].

Foot process effacement is a classical sign of podocyte injury that correlates with the onset of proteinuria, underscoring the importance of these cells as the final size-selective filtration barrier to albumin. As podocytes have limited capacity for repair or regeneration, effective glomerular function is highly sensitive to podocyte number and glomerular filtration surface area[216]. Podocytes are therefore highly attractive therapeutic targets to treat refractory congenital nephrotic syndromes (such as the Finnish type)[231].

In immortalized mouse and human podocytes (AB8/13 cells), activation of TNFα led to increased vascular cell adhesion protein 1 (VCAM-1) expression[232]. Anti-VCAM-1-coated lipidic NPs were used to deliver the potent immunosuppressant rapamycin to these activated podocytes, which inhibited cell migration[232]. Similar synthetic lipidic systems, such as synthetic, amphiphilic, interactive transfection reagent (SAINT), have also been used to package therapeutic proteins such as the large GTPase dynamin or antibodies, and were taken up by podocytes and fibroblasts[233]. The utility of this approach remains to be validated *in vivo*.

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We have shown that polymeric NPs targeted to the neonatal Fc receptor (FcRn) effectively transport insulin across the intestinal barrier[143,144]. The FcRn signalling pathway is involved in the vectorial transcytosis of IgG and protects IgG and albumin from degradation[234]. FcRn is also expressed in podocytes, where it prevents IgG from clogging the glomeruli and controls albumin excretion[234]. NPs that are targeted to FcRn, are taken up via endocytosis, and are capable of releasing their payload from endosomes (for example via pH-sensitive chemistries or other ligands that can cause endosomolysis) might prove an effective means to deliver drugs into podocytes.

The glomerular endothelium and podocytes express several integrin receptors including integrin αvβ3. Activation of integrin αvβ3 induces urokinase plasminogen activator surface receptor signalling in podocytes, which in turn leads to foot process effacement and loss of urinary protein[235]. In diabetic nephropathy, hyperglycaemia stimulates the secretion of integrin αvβ3 ligands such as vitronectin by vascular cells, leading to the activation of αvβ3 integrins[235]. Blocking the ligand occupancy of αvβ3 with the $F(ab')_2$ fragment (obtained by pepsin digestion of an immunoglobulin) of an antibody directed against the extracellular domain of the integrin β3 subunit inhibited the development of diabetic nephropathy in diabetic pigs and rats[236,237]. Using NPs targeted to integrin αvβ3 that transport siRNA payloads directed against inflammatory pathways in podoctyes could be a promising approach to treat podocyte injury in kidney disease. Inorganic NPs surface modified with a cyclic RGD targeting motif (a sequence originally found in fibronectin) and targeted to integrin αvβ3, underwent receptor-mediated uptake and accumulated in vesicle-like structures in 2D podocyte cultures and whole glomeruli *ex vivo*[238]. The identification of podocyte-specific targets, coupled with the identification of specific upregulated pathways that underlie podocyte function in health and disease, will enable the creation of nanomedicines targeted to this important cell type for clinical translation and treatment of glomerulopathies.

Mesangial cells

Mesangial cells function as microvascular pericytes and maintain the structural architecture of the glomerular capillary (FIG. 2). They are primarily responsible for glomerular matrix growth and homeostasis, regulation of filtration surface area, and clearance of apoptotic cells or immune complexes near glomerular capillaries[183].

Increased matrix production by mesangial cells and glomerulosclerosis are hallmarks of kidney disease and mesangial cell dysfunction is a key event in diabetic nephropathy. Hyperglycaemia induces excess production of reactive oxygen species, growth factors, and cytokines in mesangial cells[183]. Targeted NP-mediated drug delivery to mesangial cells would therefore be useful to treat various kidney diseases. NPs have the potential to diffuse and accumulate for prolonged periods in the mesangium if they can successfully cross the glomerular endothelial barrier. In fact, PEGylated gold NPs (with negative surface charge and variable PEG lengths) can be delivered to the kidney mesangium depending on their size[167]. Intravenously injected 75 nm NPs effectively targeted the kidney mesangium, whereas NPs >130 nm were abundant in the liver and spleen but not detected in the kidney. In this study NPs of ~130 nm in diameter provided an '*in vivo* calibration' for the size of

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glomerular endothelial pores in physiologically relevant conditions as values for this threshold are usually derived from direct measurements of transmission and scanning electron microscopy images, for which sample preparation (repeat dehydration) might lead to pore shrinkage[167]. Cyclodextrin-containing siRNA NPs quickly accumulated in the GBM and also effectively delivered siRNA to mesangial cells to silence enhanced green fluoescent protein (EGFP) expression in the glomeruli of transgenic mice[167,239].

Antibody-coated immunoliposomes effectively target mesangial cells. For example, NPs coated with anti-integrin-α8 monoclonal antibodies targeted mesangial cells in lupus glomerulonephritis in mice[105]. Similarly, NPs coated with OX7, an anti-Thy-1 membrane glycoprotein (Thy-1) monoclonal antibody, targeted the rat mesangium in mesangial proliferative glomerulonephritis[127,131]. Interestingly, the liposomes investigated in these studies were 130–170 nm in size, suggesting that such NPs can cross the glomerular basement membrane via surface ligand targeting (unlike non-targeted NPs)[131]. For optimal therapeutic effects, targeted drug delivery systems such as immunoliposomes can be used to deliver high intracellular concentrations of molecules that usually have severe systemic toxicities (glucocorticoids, cyclophosphamide, azathioprine, fatty acids, anticoagulants or antithrombotic agents) to mesangial cells using NPs conjugated to antibodies directed against antigens expressed on mesangial cells.

Tubular epithelial cells

The proximal tubules both secrete waste products and reabsorb useful nutrients into and from the urine[122]. Two barriers are involved in those processes: the capillary endothelial cells at the basolateral side of the proximal tubular cells and the tubulointerstitium between the capillaries and tubular cells[122] (FIG. 2). Renal peritubular capillaries have fenestrations (60–70 nm wide), which are covered by a diaphragm (3–5 nm thick)[122]. In order to cross the tubulointerstitium, particles need to be smaller than the size of this diaphragm (<5 nm), and positively charged as the fenestrae are negatively charged owing to the presence of heparin sulfate[122]. Furthermore, the tubulointerstitium also contains fibroblasts and dendritic cells within an extracellular matrix consisting of proteoglycans, glycoproteins, fibrils, and interstitial fluid[122]. The tubular epithelium is highly susceptible to injury, which leads to tubular cell loss and sublethal proinflammatory and fibrogenic injuries[240]. Fibrosis is the downstream pathological manifestation of chronic kidney disease and is strongly correlated with disease progression. Hence, NPs designed to deliver drugs that prevent cell death, reduce renal fibrosis and inflammation, and/or promote the regeneration of tubules could be highly valuable[241].

An optimal strategy to reduce fibrosis would be to target proximal tubular epithelial cells, as these cells play an important role in the pathogenesis of tubulointerstitial fibrosis. Targeted drug delivery strategies to inhibit pathways that are activated in disease with TGFβ kinase inhibitors, p38 mitogen activated protein kinase (p38 MAPK) inhibitors, Rho-associated kinase (ROCK) inhibitors or PDGF receptor kinase inhibitors, for example, could slow or even prevent progression to renal disease[122].

As proximal tubule cells accumulate siRNA, several RNAi-based strategies have been proposed to treat acute kidney injury. NP-mediated delivery of siRNA can increase the

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accumulation of siRNA molecules within proximal tubule cells and facilitate combination treatments or controlled-release modes of action[242,243]. As mentioned above, carbon nanotubes with siRNAs against *Trp53*, *Mep1b* and *Ctr1* reduced renal injury, fibrosis and immune infiltration in a cisplatin-induced model of acute kidney injury[202]. Although the pharmacokinetic profiles of the carbon nanotubes were comparable in mice and primates, suggesting that this therapy might be amenable to humans, the potential advantages of siRNA delivery using these NPs should also be investigated in the clinic.

A 2015 report showed that 400 nm NPs localize preferentially in the kidney compared to other organs, and selectively accumulate in the renal proximal tubules[244]. This characteristic suggests that antifibrotic compounds could be delivered directly to proximal tubular cells, which would increase renal specificity and minimize adverse and non-targeted effects; however, further studies are required to confirm these findings.

The effective targeting and optimal transport strategies to deliver xenobiotics and various drug conjugates (with polymers and/or proteins) to tubular cells have been discussed at length elsewhere[122]. The role of proximal tubular cells transporters, which are involved in the tubular reabsorption and secretion of small molecules, in the efflux of colloidal or nanosized drug-delivery systems remains to be elucidated. Large macromolecular structures are highly unlikely to be transported into tubular cells via this mechanism in healthy states; the transport of NPs and large macromolecules into tubular epithelial cells in disease states needs to be further investigated.

Catechol-derived, low-molecular-weight chitosan NPs were developed and investigated for the treatment of renal fibrosis in a 2014 study[120]. This self-assembled nanocomplex, designed to selectively release its pay-load inside cellular endosomes for maximal specificity, was specifically absorbed in the proximal tubules in mice. Intravenous delivery of the active anti-fibrosis compound emodin with these NPs attenuated fibrotic progression in a murine model of renal obstruction. Multi-targeted kinase inhibitors, and targeted delivery with nanomedicines, might also achieve antifibrotic effects. For example, sunitinib (a multi-targeted kinase inhibitor) has been evaluated for targeted drug delivery (conjugated to the kidney-specific carrier lysozyme)[245], indicating that lysozyme could be utilized as a targeting ligand. A 2015 study investigated the effects of delivering an exogenous microRNA (miR-146a) using polyethylen-imine NPs in a mouse model of renal fibrosis induced by unilateral ureteral obstruction[246]. These positively charged NPs markedly reduced renal fibrosis by inhibiting inflammation via the suppression of TGFβ1 and Smad mRNA expression, which are responsible for the transcription of profibrotic genes.

A 2015 study showed that 5 nm dextran-based NPs and similar-sized poly(amidoamine) dendrimer NPs were filtered and internalized by renal tubular epithelial cells in a dose and time-dependent manner[137]. The administration of dextran NPs increased cellular albumin endocytosis and megalin expression but did not affect AQP-1 expression, as measured by dose-dependent urinary output. Similar effects were observed with the dendrimer NPs, which, in addition, led to an increase in clathrin expression. No major renal detrimental effects of dendrimers were reported, although changes in proximal tubule endocytosis and

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protein cellular expression levels suggest that these parameters should be monitored as researchers develop nanodrugs of various compositions and sizes targeted to the kidney.

Renal cell carcinoma

Renal cell carcinoma is the most lethal urologic cancer and affected patients have limited treatment options[247]. Over the past decade, cytokine-based therapies that target VEGF and mTORC pathways have improved the treatment of this disease; however, resistance mechanisms exist, which could be mitigated with combination therapies.

Numerous liposomal strategies for drug delivery in renal cell carcinoma have been investigated in preclinical studies, but only a few clinical studies have been carried out[248–259]. The majority of these studies administrated standard renal disease therapies and used antibody- targeting strategies. PEGylated-liposomal doxorubicin (Doxil) was used to treat patients with refractory renal cell cancer in a phase II clinical trial but had no efficacy and was mildly toxic[257]. Polymeric NPs have also been investigated in preclinical models for the delivery of the anti-angiogenic compound tetraiodothyroacetic acid to tumour cells in the chick chorioallantoic membrane and in xenografts in mice[260].

Targeted therapies and immunotherapies are proving effective for the treatment of metastatic renal cell carcinoma, and several approved targets (such as new immunomodulatory monoclonal antibodies targeting the CTLA-4 or PD-1 pathways) are already available, although the clinical benefits of these new therapies compared to established treatments (tyrosine kinase or mTOR inhibitors) remains to be shown[261]. Nevertheless, nanomedicine-based approaches are of interest for the targeted delivery of chemotherapies and nanovaccines and their development will require further studies.

Nanotechnology has been widely applied to cancer therapy, and much has been learned regarding the discovery, manufacturing, and preclinical and clinical development of nanomedicines from several types of nonrenal cancer. We can extrapolate and infer from this base of knowledge to improve the design of renal carcinoma nanomedicines. Despite the fact that the safety profiles of most clinically validated NP platforms are superior to those of the parent drug, the nanomedicine does not improve survival when compared head-to-head with the parent drug. This absence of effect might be due, in part, to disease heterogeneity and variations in the degree of the enhanced permeability and retention (EPR) effect, according to which NPs preferentially accumulate in tumour tissues. The EPR effect has been suggested to have a central role in nanomedicine delivery to tumours, underscoring the need to select patients that are most likely to benefit from nanomedicines[211,262–266]. This effect has been widely investigated in preclinical models but few clinical studies have characterized it in humans or assessed its relevance to therapeutic intervention[211,262–264]. Furthermore, as understanding of the contribution of the perivascular tumour microenvironment to the penetration depth and uptake of NPs within tumours and into cancer cells has grown, we have progressively recognized that the EPR effect is complex and dynamic. The degree of tumour vascularization, inter-patient genetic variation, and tumour type and aggressiveness are all factors that contribute to this complexity[211]. Thus, stratification of patients with cancer according to tumour type and characteristics in relation

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to the likeliness of NP accumulation might be necessary to maximize the benefits of NP-mediated drug delivery through the EPR effect[266–268].

In the past two years, preclinical efforts and preliminary clinical studies have begun to explore companion MRI-active nanodiagnostic molecules to dynamically assess response to nanomedicines[265,266]. Companion diagnostics could also prove valuable to assess NP retention, targeting, and biodistribution in the nephron using existing clinical modalities such as ultrasound, MRI, CT, and radiographs. These methods could be used for the assessment of NP accumulation within renal cell carcinomas[269].

In vitro systems to assess NP efficacy

Controlled and optimized *in vitro* and *ex vivo* biological assays are required to test renal nanomedicines effectively. Traditional 2D cell culture models do not support complex tissue functions such as epithelial-to-mesenchymal transition or accurately predict the *in vivo* effects of a drug.

Microfluidics and kidney-on-a-chip

Organs-on-chips are built using microfluidic devices in which multiple cell types are cultured in 2D under continuous perfusion. These systems support tissue patterning *in vitro* and have been successfully used to study the molecular basis of tissue morphogenesis[270]. They also enable the screening of nanomedicines under physiological conditions[270–272] (FIG. 3). For example, exposure of epithelial monolayers of collecting duct cells and proximal tubule cells to an apical fluid shear stress mimicking the rate found in living kidney tubules in a microchip resulted in enhanced epithelial cell polarization and primary cilia formation and provided an environment resembling that found *in vivo*, which is amenable to toxicity studies[272]. In this system, fluid shear stress might have induced actin cytoskeletal rearrangements, resulting in a cellular phenotype akin to that found in human kidneys. A variety of animal and human renal cells cultured in these conditions had a toxicity response similar to that found *in vivo*. Such systems also effectively recreated epithelial-to-mesenchymal transition, which mediates renal interstitial fibrosis[272–277], and facilitated the measurement of P-glycoprotein ATP-binding cassette membrane (PGP, also called multidrug resistance protein 1 transporter activity in response to chemotherapy). Indeed, kidney tubular epithelial cells had more effective PGP efflux activity in baseline conditions under shear flow than without flow, which more closely resembles the *in vivo* environment than measures with traditional culture systems[272].

Lessons from such studies could prove valuable for screening kidney-targeted drugs and assessing nanodrug nephrotoxicity and efficacy in a more quantitative and dynamic manner than traditional culture systems[271,272,278].

3D culture systems

Advances in induced pluripotent stem cell technologies and in the understanding of kidney morphogenesis have enabled the production of mature kidney cells *in vitro* in 3D structures called organoids[279–281] (FIG. 3). Organoids are self-organizing 3D tissues that can be grown

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from embryonic stem cells, their synthetic induced pluripotent stem cells or organ-restricted adult stem cells[282,283].

The kidney includes more than 20 specialized cell types and is an organ with complex structural design, which is not accurately portrayed using traditional 2D culture systems. In contrast, kidney organoids can be grown with more complexity, including more than 500 nephrons and defined glomeruli with Bowman capsule and podocytes, connected to proximal tubules[279,284].

One of the next areas of development for kidney organoids is to achieve urine and blood filtration *in vitro*, which will require a complete kidney structure with a blood system connected to glomerular capillary loops. Establishing patient-derived organoids is also an exciting avenue to develop true personalized medicines, whereby drug response can be predicted according to the patient's genetic makeup. Furthermore, kidney organoids can facilitate testing of regenerative medicines, combination therapies, gene editing therapies and nanomedicine drug delivery, as they provide an environment akin to that found in the kidney but with the ease of manipulation of an *in vivo* system[285]. The toxicity of dendrimer NPs was assessed in a proximal tubule organoid, which expressed toxicity markers previously reported *in vivo*[286]. Such functioning proximal tubule cultures provide an environment similar to that found *in vivo* to assess the nephrotoxicity of nanomedicines and their cargos as they express multiple drug transporters, akin to their *in vivo* counterparts[287].

Challenges to clinical translation

Clinical grade synthesis and screening

NPs are often composed of several components and can have a range of biophysical and chemical properties (size, charge, drug payload, release kinetics and surface targeting ligand) that require screening in a systematic and parallel fashion, while ensuring reproducible synthesis of libraries with such distinct features. Bulk techniques do not always generate uniform NPs even if they can be scaled to yield multi-kilogram batches of particles suitable for clinical development and commercialization[288]. Microfluidics have improved the synthesis of a variety of NP systems and enabled the controlled synthesis and high-throughput screening of NP libraries[288,289]. Microfluidic technologies have the potential to become a platform for rapid self-assembly of NPs with a narrow size distribution, tuneable physical and chemical characteristics, and low batch-to-batch variability[290–294]. Other techniques such as PRINT have also been used to synthesize uniform NPs with precise control of size, shape, chemical composition, drug loading, and surface properties[192,193]. Analogous to high-throughput drug discovery screening, these technologies could also help to streamline a controlled and high-throughput investigation of kidney NP structure–activity relationships.

Chemistry, manufacturing and controls (CMC) and good manufacturing practice (GMP) requirements must continuously be met as NP technologies transition from preclinical to clinical development, then to commercialization and throughout the market life of the product. Current pharmaceutical manufacturing unit operations that generate first-generation NPs (liposomes and polymeric NPs) meet such demands; however, producing more complex

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nanomedicines, such as those that incorporate targeting ligands, multidrug combinations, or multi-stage, stimuli-responsive, or theranostic systems, can create additional CMC requisites and require the adaptation of current manufacturing unit operations. The large-scale production of high-quality complex NPs also needs to be addressed. PRINT technology facilitates reproducible fabrication of NPs[193], but production at the kilogram scale remains to be demonstrated. Alternative microfluidic technologies such as the coaxial turbulent jet mixer, which provides NP homogeneity, reproducibility, and tuneability, have also been designed and developed for large-scale production of polymeric NPs[295]. In the future these methodologies can be used to scale-up clinical-stage nanomedicines targeted to the kidney.

In vivo testing

Investigations into *in vivo* animal models, which can sometimes accurately mimic human diseases, are also important. Some studies have shown pharmacokinetics scaling across species (including humans) for different nanotherapeutics[64,86,296,297]. For instance, the AUC of polymeric NPs containing camptothecin (CPT) scaled linearly with CPT dose per m^2 across species (mice, rats, dogs, and humans), suggesting that NPs behave in a similar way in animals and in humans[287]. However, interspecies discrepancies exist for albumin binding of CPT (with higher binding affinity found for human albumin), and should be taken into account when considering the pharmacokinetics of the free drug in humans as this property might be important in investigations of NP versus free drug efficacy. Furthermore, NP biodistribution studies in patients are not feasible in most cases. The discrepancies between pre-clinical and clinical models must therefore be considered for NP development and animal models that more closely mimic the heterogeneity and anatomical and histological features of human kidney disease are needed[298,299].

Conclusions and Perspectives

As the field of nanomedicine rapidly expands, with several NPs already marketed and many undergoing clinical trials, our accumulated experience and the clinical successes to date form a framework for the creation of the next generation of renal nanomedicines. Refining the generation of nanomaterials with tuneable biophysical properties could yield NPs with highly controllable architectures for kidney binding and retention as well as uptake within important cell types such as podocytes. Fortunately, in the past few years, numerous studies of NP or nano-sized colloids originally intended for tumour targeting have serendipitously yielded important discoveries relevant to kidney targeting and selective renal accumulation. These, in turn, raise important correlations between the structure and the activity of NPs according to their design and biophysical and chemical properties as a function of kidney retention and targeting. We believe that now is an ideal time for collaborative initiatives between nephrologists and nanotechnologists to pioneer and establish promising nanomedicine approaches, with appropriate selection of targeting and therapeutic parameters for the diagnosis and treatment of renal disease.

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Glossary

Nanoparticles
Nanoscale particles with modifiable shape and charge capable of carrying a specific payload (drugs, diagnostic molecules etc.).

Colloid
Substance formed by a non-crystalline material (here nanomaterial) of either natural or synthetic origin dispersed in a solution.

Organ-on-a-chip
Microfluidic devices used to culture living cells under continuous perfusion in micorometer-sized chambers; they are primarily used to reproduce the physiological functions of tissues and organs as closely as possible.

PEGylation
Attachment of polyethers to the surface of nanoparticles in order to minimize unwanted interactions with their biological surroundings.

C_{max}
Maximal serum concentration of a drug or nanoparticle achievable after administration.

Top-down fabrication method
Synthesis of a structure by etching or removal of material from a template or substrate to achieve specific shapes or sizes.

Particle replication in non-wetting templates
Fabrication technique whereby a pre-particle solution is distributed in a mould with nanosized cavities to generate nanoparticles with precisely defined shape, size, composition and surface properties.

Aspect ratio
Ratio of the width to the height of a nanoparticle.

Enhanced permeability and retention (EPR) effect
Describes the accumulation and retention of colloidal nanoparticles within tumour tissue as a result of increased endothelial gap junction distances due to heterogeneous vessel formation and growth.

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Key points

- Oncology has benefited greatly from nanotechnology research and investment, yielding important insights that are applicable to kidney nanomedicines
- Nanocarriers have the potential to improve the pharmacokinetics, biodistribution, toxicity, and efficacy of drugs
- Delivery and retention of nanomedicines in the kidney is challenging and requires a deep understanding of the renal barriers and effective engineering of nanoparticle size, shape, and surface chemistry
- To improve drug efficacy and minimize systemic toxicity, nanomedicines can be targeted to distinct kidney cell types and/or to extracellular matrix components such as the glomerular basement membrane
- Nanocarriers can deliver drugs, proteins, peptides and nucleic acids, and can facilitate the targeted delivery of genes or RNA interference molecules to treat kidney disorders for which the efficacy of current treatments is limited
- Organ-on-a-chip kidneys can streamline the simultaneous screening and testing of nanomedicines in environments that mimic the kidney and accelerate translation of kidney-specific therapeutics

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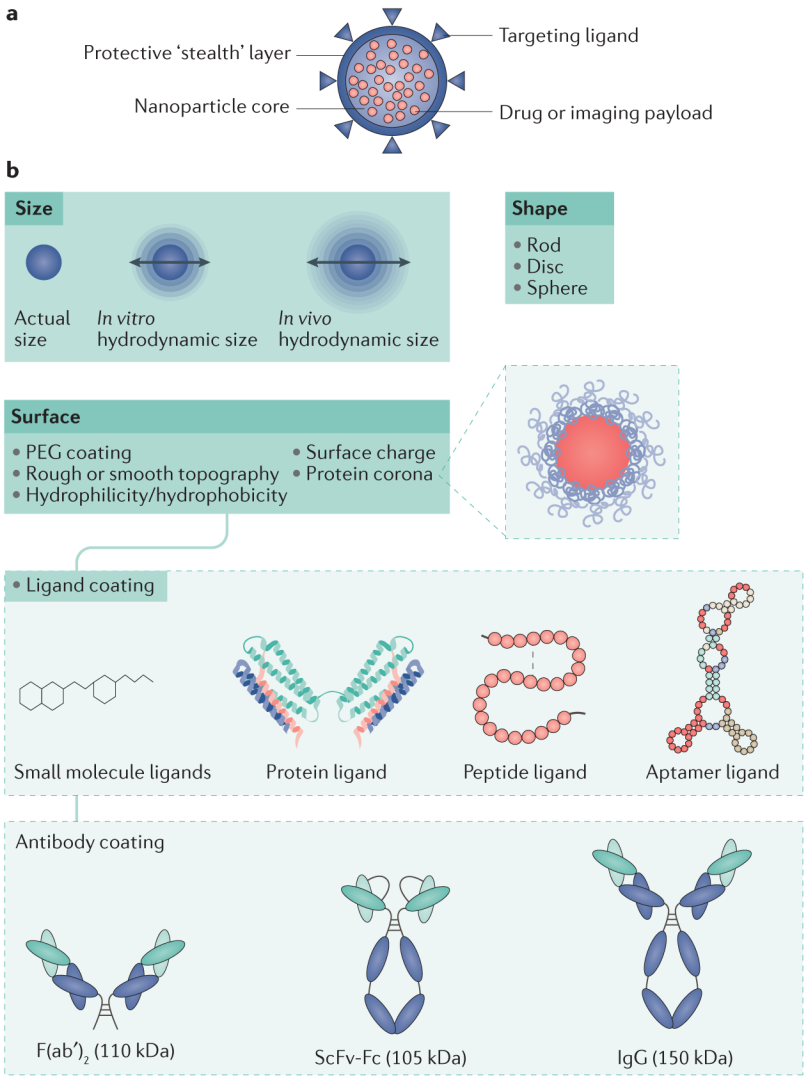

Figure 1. Nanoparticle composition and features

a | Nanoparticles are composed of a core (payload) encapsulated in a protective layer. The surface can be modified to limit the interactions of the particle with its environment. **b** | Several parameters such as size, shape and surface modification contribute to the biological and physicochemical properties of nanoparticles. The investigation of such properties is crucial to achieve the translational application of nanomedicines for kidney disease therapy. PEG, polyethylene glycol; ScFv Fc, single chain variable fragment constant fragment.

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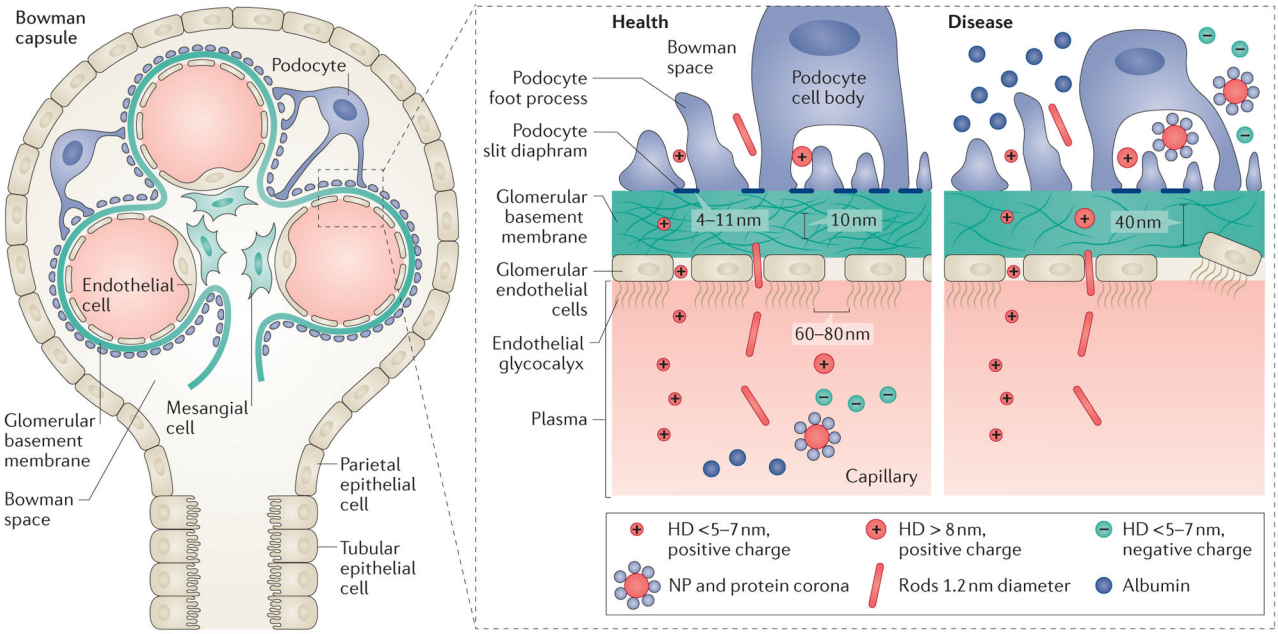

Figure 2. The kidney glomerulus and the glomerular basement membrane in health and disease

The glomerular filtration barrier consists of glomerular endothelial cells, the glomerular basement membrane, and podocytes. All solutes and molecules with a molecular weight less than that of albumin (68 kDa) and a hydrodynamic diameter (HD) <5–7 nm can pass this barrier. The glomerular filtration barrier is negatively charged and in healthy states repels negatively charged proteins (such as albumin) and nanoparticles (NPs). In disease, podocyte effacement leads to the breakdown of the barrier and proteinuria. The presence of leaky and abnormal fenestrae can aid the accumulation of large and/or charged nanodrugs in the Bowman space.

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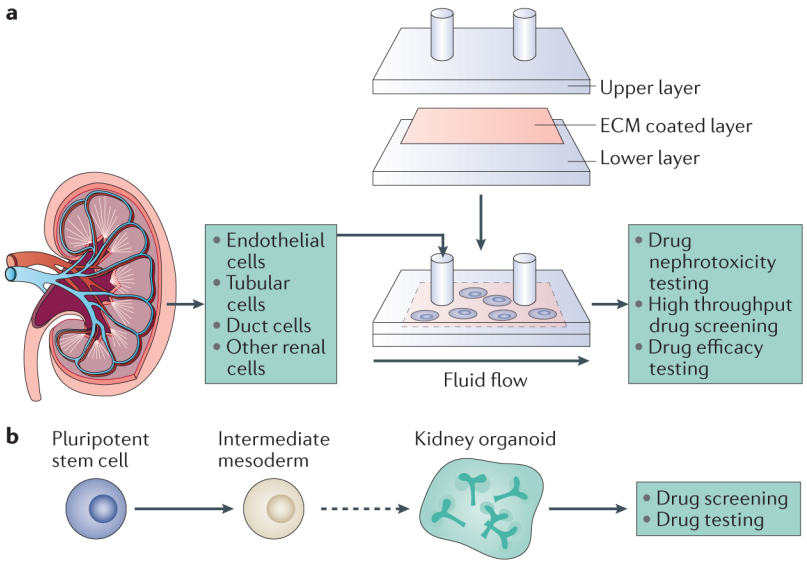

Figure 3. *In vitro* systems to test nanoparticles

a | Different types of primary renal cells can be seeded on a porous membrane coated with extracellular matrix (ECM) and cultured in microfluidic devices that mimic physiological conditions with apical fluid shear stress. These devices enable easy fluid sampling and addition of nanodrugs, which facilitates the high throughput testing of nanodrug nephrotoxicity and efficacy. **b** | Pluripotent stem cells can be induced to differentiate *in vitro* into mesoderm and renal organoids: 3D structures that contain several renal cell types. Organoids can be used as a platform to screen and test nanodrugs.

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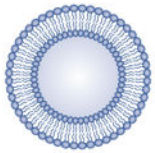
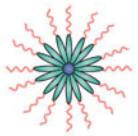
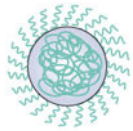
Table 1

Clinical use of nanoparticles

Nanoplatform	Composition	Size	Examples of nanoparticles applied to the clinic	Disease indication	Active pharmaceutical ingredient	Clinical status in 2016	Refs
Liposomes 	Spherical lipid bilayer with an aqueous core	~100–200nm	Non targeted: Doxil	HIV-related Kaposi sarcoma, ovarian cancer, multiple myeloma	Doxorubicin	Approved	29–45
			Targeted: MM 302	$Her2^+$ breast cancer	Doxorubicin	Phase II	63
			Non targeted, stimuli responsive: ThermoDox	Hepatocellular carcinoma	Doxorubicin	Phase III	300
			Non targeted, combination therapy: CPX 351	Acute myeloid leukemia	Cytarabine and daunorubicin (5:1)	Phase III	66
Polymer–drug conjugates 	Nanosized colloids generated by covalent conjugation of drugs to pendant groups on polymer backbones	~5–20 nm	Non targeted: Zinostatin	Liver cancer	Styrene maleic anhydride	Approved	61–62
Polymeric micelles 	Lipids or polymers with a hydrophilic– hydrophobic core–shell structure	~10–100 nm	Non targeted: Genexol PM	Breast cancer, non-small-cell lung cancer	Paclitaxel	Approved	52–53
Polymeric 	Solid core, formed by the self-assembly of degradable polymers	~50–100 nm	Non targeted: Livatag	Liver cancer	Doxorubicin	Phase III	27
			Targeted: BIND 014	Active targeted delivery to non-small-cell lung cancer, metastatic castration resistant prostate cancer, other metastatic cancers and solid tumours	Docetaxel	Phase II	64

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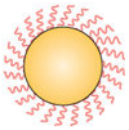
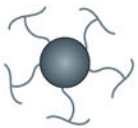
Nanoplatform	Composition	Size	Examples of nanoparticles applied to the clinic	Disease indication	Active pharmaceutical ingredient	Clinical status in 2016	Refs
Protein-based 	Albumin-based delivery platform	~130 nm	Non targeted: Abraxane (Nab paclitaxel)	Breast, lung, and pancreatic cancer	Paclitaxel	Approved	46–51
Dendrimers 	Symmetrically branched polymeric macromolecules synthesized using polyamides	<10 nm	Non targeted: Vivagel	HIV infection	NA (anionic G4-poly(L-lysine)- type dendrimer displaying 32 napthalene disulfonate surface groups)	Phase III	67–69
Gold-based 	Spherical, rod or shell based colloids made from the reduction of $HAuCl_4$	~27 nm	Non targeted: CYT-6091	Solid tumours	Tumour necrosis factor	Phase I	70–72, 80
Silica based 	SiO_2 NPs	20–200 nm	Non targeted: AuroLase (silica core with a gold nanoshell)	Head and neck cancer, lung cancer	NA (laser ablation)	Pilot study	74,80, 81
Iron oxide based 	Crystalline nanoparticles of $Fe_3O_4/\gamma\text{-}Fe_2O_3$	10–20 nm	Non targeted: Nanotherm	Glioblastoma	NA (heat)	Approved	77

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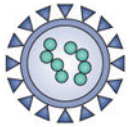
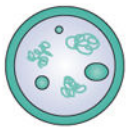
Nanoplatform	Composition	Size	Examples of nanoparticles applied to the clinic	Disease indication	Active pharmaceutical ingredient	Clinical status in 2016	Refs
Hafnium oxide based 	HfO_2 NPs	~50 nm	Non targeted: NBTXR3	Adult soft tissue sarcoma, head and neck cancer	NA (Radiotherapy)	Phase I	78,79
Viral 	Viral and virus-like nanoparticles	20–50 nm	Non targeted: Oncolytic poxvirus JX-594	Stimulation of anti-tumour immune response	Granulocyte colony-stimulating factor	Phase I, Phase II	92,93, 94
Exosomes 	Naturally secreted cell-derived vesicles	30–100 nm	Targeted or non targeted: various biologically derived nanosized exosomes	Immunotherapy of melanoma, colon cancer, diabetes, wound-healing, oral mucositis, gastric cancer (biomarker), oropharyngeal cancer (biomarker), thyroid cancer (biomarker)	Various biological payloads	Phase 0, Phase I, Phase II	95–98, 301
Carbon-based 	Polycyclic aromatic hydrocarbons	>1 μm	Targeted or non targeted: Nanospheres, nanotubes, nanosheets	Breast cancer, lung cancer, other solid tumours	Various poorly soluble therapeutics in high concentrations	Preclinical	99–103