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Nanomedicine: controlling nanoparticle clearance for translational success

Geyunjian Harry Zhu,[1] Alex B.C. Gray,[2] and Hirak K. Patra[2,*]

Achieving complete nanoparticle (NP) clearance is a key consideration in the design of safe and translatable nanomedicines. Renal-clearable nano formulations must encompass the beneficial nanoscale functionalities whilst exhibiting clearance profiles like those of small-molecule therapeutics. Recent developments in the field have enabled the growth of novel renal-clearable NPs with transformable sizes that take advantage of alternative clearance mechanisms to achieve controlled and efficient renal excretion to improve potential clinical translation.

Why do we care about NP clearance?

Numerous NP-based drug delivery systems have been developed to achieve targeted drug delivery to tumours and other diseased sites. Nano-systems achieve targeted delivery via either passive or active targeting. Passive targeting exploits the **enhanced permeability and retention (EPR)** (see Glossary) effect that causes nanosized materials to preferentially accumulate in tumours due to their leaky vasculature and lack of lymphatic drainage [1]. By contrast, NPs can actively target tumours by carrying carefully selected ligands that are recognised by cell-surface receptors commonly expressed on tumour cells. However, only <1% ID (injected dose) of administered NPs arrive at their intended target (e.g., a solid tumour) [2]. Body clearance mechanisms for foreign objects and the numerous biological *in vivo* barriers probably account for such low targeting efficiency. The remaining 99% ID can potentially accumulate in unintended healthy organs and tissues, competing with the tumour for uptake, leading to undesirable side effects. Designing NPs that support clearance could be beneficial in lowering their toxicity; however, a rapid clearance would further negate their targeting and therapeutic efficiency. It is therefore clear that accomplishing a balance is essential to producing efficacious, safe, and translatable nanomedicines. This forum article focuses on the fundamentals to consider when designing renal-clearable NPs, and highlights the recent trends in the field.

What do we know about NP clearance?

Like other therapeutics, NPs must be metabolised or cleared from the body to avoid accumulation and resultant toxicity. The two main clearance mechanism for NP expulsion are the hepatic and renal pathways (Figure 1). In the hepatic route, NPs are captured by the mononuclear phagocyte system (MPS) or tissue-resident phagocytes, leading to their accumulation in the liver and spleen. Subsequently, NPs are subject to biliary elimination and excretion via the stools. In the renal pathway, NPs are directly cleared via renal filtration and are expelled in the urine [3]. The hepatic route involves the extensive breakdown of nanomaterials by hepatocytes, whilst renal excretion is more rapid; this prevents the lengthy and unpredictable interactions between NP and host, making renal excretion the more desirable clearance pathway [4].

To pass through the kidney filtration barrier, nanomaterials must traverse three layers in the glomerular membrane: the endothelium with fenestrae (70–90 nm), meshwork glomerular basement membrane (with pores 2–8 nm), and epithelium with filtration slits (4–11 nm) [5]. The structural features of the glomerular membrane determine its size-dependent filtration selectivity. It is worth mentioning that the size of NPs almost always increases once administered *in vivo* due to the adsorption of a protein corona onto the particle surface. Hence it is the '*in vivo* size' rather than the physical size that determines the renal clearance of the NPs. Renal clearance is also determined by the size, shape, and surface chemistry of the NP. For instance, positively charged particles are cleared more rapidly via the renal route than negatively charged particles [6].

Thus, to undergo primarily renal clearance, NPs should be smaller than the **kidney filtration threshold (KFT)** of ~6 nm while simultaneously avoiding sequestration by the MPS. NP recognition by phagocytes is mediated through protein opsonisation. This means that the formation and composition of an NP's corona greatly determines its uptake by the MPS [7]. The conventional approach to reducing phagocyte recognition involves the use of **antifouling** and hydrophilic polymers, such a polyethylene glycol (PEG) and zwitterionic polymers, to cloak the surface of the NPs, whereas true **bioinvisibility** requires the presence

Glossary

α-phase half-life: the time it takes for the plasmon concentration of a drug to drop by half due to the rapid redistribution from the central to the peripheral compartments.
Antifouling: the ability to prevent/alter the accumulation and attachment of proteins.
β-phase half-life: the time it takes for the plasmon concentration of a drug to drop by half due to metabolism and excretion.
Bioinvisibility: the state of an object that prevents detection by a host's immune system.
Enhanced permeability and retention (EPR): the mechanism by which nanoparticle-based drug delivery systems accumulate in tumours due to their enhanced vasculature permeability and reduced lymphatic drainage.
Kidney filtration threshold (KFT): the size limit at which molecules/particles can undergo renal filtration. It is believed to be <6 nm.

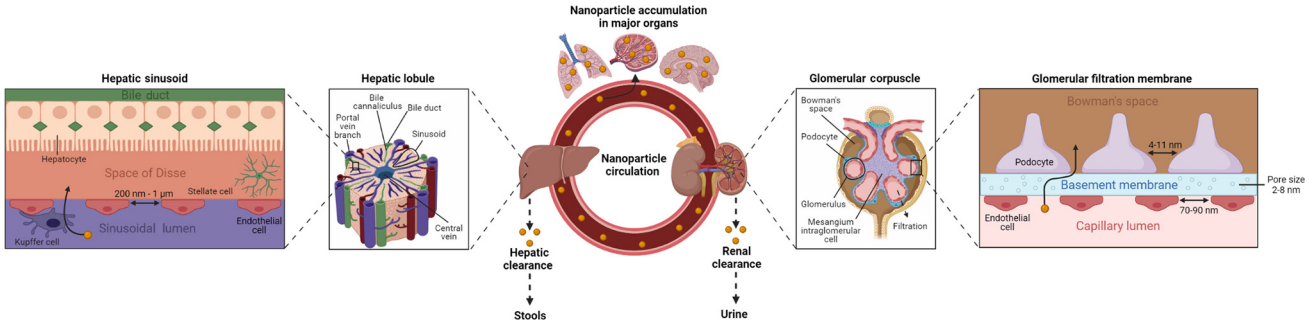

Figure 1. Nanoparticles (NPs) are cleared from the body via two main clearance pathways: hepatic and renal. The hepatic pathway relies on components of the mononuclear phagocyte system (MPS), such as Kupffer cells, to recognise and phagocytose NPs. The NPs' protein corona influences their susceptibility to recognition by the MPS. Once they are captured in the liver or spleen, they can be broken down and slowly excreted through the biliary pathway. This mechanism involves long NP–host exposure times and the risk of accumulation in resident macrophages. NP accumulation in major organs poses the risk of toxic side effects. By contrast, the renal pathway offers a safer clearance route by way of renal filtration which avoids extended interaction with the host. However, the size-dependent barriers in the glomerular filtration membrane restrict the sizes of NPs that can be cleared via this route.

of self-identifying proteins to achieve eukaryotic mimicry [8]. Both size and surface properties should be considered when designing renal-clearable NP systems.

Can efficient renal clearance be achieved without compromising targeted delivery?

The development of an NP-based system that enables their renal clearance has been largely driven by safety concerns. It has been reported that rapid clearance by either renal or hepatic pathways decreases nonspecific uptake by major organs and tissues, resulting in a reduced risk of toxicity and better tumour-to-background ratio [4]. However, the targeting efficiency should not be compromised excessively by renal clearance. Targeted delivery and renal clearance appear to be somewhat contradictory concepts because avoiding renal excretion is believed to be a prerequisite of elongated circulation time according to the EPR effect. Nonetheless, efficient renal clearance does not necessarily equate to fast renal excretion**.** In an ideal scenario, NPs should achieve elongated circulation for consecutive days, allowing optimal targeting opportunities whilst being gradually excreted by the renal pathway to avoid the risks associated with hepatic clearance. Liu *et al.* demonstrated the enhanced yet steady renal clearance of glutathione-coated MoS_2 (MoS_2-GSH) nanodots compared with larger, more conventional MoS_2 nanoflakes; MoS_2-GSH nanodots (H_D 7.5 nm) exhibited <50% ID in the urine 24 h postinjection, whilst efficient renal clearance (~70% ID) was reported over 1 week [9]. Similarly, an ultrasmall coordination nanodot (H_D 5.6 nm) exhibited only ~32% ID renal clearance after 12 h, but 85.68% ID at 7 days [10]. Both of these NP systems were able to circulate within the organism for a relatively long time, allowing time for targeted delivery while simultaneously achieving high renal clearance rates over time.

How can the long circulation time and efficient renal clearance be accomplished simultaneously?

More recently, NPs with transformable sizes that can exploit the renal clearance pathway, allowing for both long circulation times and efficient clearance, have been reported. A promising design strategy involves the fabrication of large nanoclusters (10–100 nm) which over time disassemble into smaller nano building blocks with sizes below the KFT. Several systems have achieved improved renal clearance using this technique (Table 1). However, other approaches exhibiting transformable sizes have failed to display efficient renal clearance. Both biodegradable liposomes (100 nm) coated with small gold NPs (AuNPs) (2–8 nm) and PEG-polycaprolactone micelles (75 nm) loaded with AuNPs (1.9 nm) for photothermal therapy and computer tomography (CT)-guided radiation therapy, respectively, failed to achieve enhanced renal excretion [11,12]. AuNP-liposomes were shown to mainly accumulate in the liver, whilst the AuNP-micelles were mostly eliminated by biliary excretion. Despite having the small building blocks, the degradable NP strategy faces some challenges: (i) the degree of disintegration needs to be high for sufficient release of renal-clearable building blocks, (ii) the assembled cluster must be degraded before being captured by the MPS, and (iii) the dissembled NPs, in addition to their small *in vitro* size, must possess suitable *in vivo* H_D and surface chemistry to efficiently pass through the glomerular filtration barrier.

By contrast to transformable NP sizes, other systems utilize an alternative renal clearance pathway which operates with a larger size threshold (Table 1). Until recently, NPs were believed to be almost exclusively excreted via glomerular filtration. Consequently renal-clearable NPs have been designed using sizes smaller than the KFT. However, recent reports have found that NPs with sizes

Table 1. NP-based systems that utilise novel strategies to facilitate and enhance their clearance via the renal pathway[a]

NP	Application	Strategy	Size (nm)	Indication of renal clearance	Refs
AuNC-GSH-peptide complexed with neutravidin	Colourimetric diagnostic platform for protease-related diseases (e.g., cardiovascular disease and cancer)	Enzyme-responsive size shrinkage facilitating GF	Complex: 11.3 NP: 1.3	~73% ID in urine 1 h p.i.	[13]
Supraparticles composed of Fe_2O_3 NPs	T1-weighted MRI contrast agent for tumour imaging	Stimuli-responsive (GSH and acidic pH) disassembly facilitating GF	Supraparticle: 15 NP: 2–3	~32% ID in kidney 20 min p.i.	[14]
Fe-AP NPs	Theranostic module for photothermal therapy and tumour imaging	Stimuli-responsive (DFO injection) disassembly facilitating GF	Complex: 65 Fe NP: 2–4	~26% ID in urine 168h p.i.	[15]
Bismuth subcarbonate NTs	Tumour-targeted computed tomography and chemoradiotherapy	Stimuli-responsive (acidic pH) NT disassembly into NCs facilitating GF	NT: 8 NC: 1.5	35% ID in urine 3 days p.i.	[16]
GAG-PLGA NPs	Preliminary study to establish framework for future systems	GAG facilitated proximal tubule secretion	NP: 133	74% ID in bladder 2 h p.i.	[17]
PEGylated Fe_3O_4 NCs	Preliminary study in novel renal clearance mechanism	Translocation through peritubular endothelium to tubular epithelial cells	NC: 140 Core: 36	Intact full-sized NPs observed in urine samples by TEM images 2 h p.i.	[18]

[a]Abbreviations: DFO, deferoxamine mesylate; GAG, glycosaminoglycan; GF, glomerular filtration; GSH, glutathione; ID, injected dose; MRI, magnetic resonance imaging; NC, nanocluster; NP, nanoparticle; NT, nanotubes; p.i., post injection; PLGA, poly(lactic-co-glycolic acid); TEM, transmission electron microscopy.

much larger than the KFT (>100 nm) can be efficiently and rapidly eliminated via tubular secretion (an alternative renal clearance mechanism). These examples, presented in Table 1, demonstrate game-changing breakthroughs for renal-clearable NPs. The previously perceived size limit can now be withdrawn as novel combinations of surface modifications allow efficient renal clearance through alternative routes.

Concluding remarks

When it comes to designing efficient renal-clearable NPs, both the kinetics (rate of the renal clearance) and the equilibrium (final percentage of NPs cleared through the renal pathway) should be considered. The ideal renal-clearable NP-based delivery system should have adequate stealthing to avoid MPS capture, ensuring that the renal pathway is the dominant clearance mechanism. At the same time, NPs should possess a short **α-phase half-life** and comparatively long **β-phase half-life**, which reflects fast distribution and long circulation for effective disease targeting by the EPR effect. We anticipate the development of systems that exhibit such pharmacokinetics to improve the clinical translation of future nanomedicines.

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Declaration of interests

The authors have no interests to declare.

[1]Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, UK
[2]Department of Surgical Biotechnology, University College London, London, UK

*Correspondence:
hirak.patra@ucl.ac.uk (H.K. Patra).

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