Trends in Molecular Medicine

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REVIEWS

Opinion

Focus on Fundamentals: Achieving Effective Nanoparticle Targeting

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Successful molecular targeting of nanoparticle drug carriers can enhance therapeutic specificity and reduce systemic toxicity. Typically, ligands specific for cognate receptors expressed on the intended target cell type are conjugated to the nanoparticle surface. This approach, often called active targeting, seems to imply that the conjugated ligand imbues the nanoparticle with homing capacity. However, ligand-receptor interactions are mediated by short-range forces and cannot produce magnetic-like attraction over larger distances. Successful targeting actually involves two key characteristics: contact of the nanoparticle with the intended target cell and subsequent ligand-mediated retention at the site. Here we propose a conceptual framework, based on recent literature combined with basic principles of molecular interactions, to guide rational design of nanoparticle targeting strategies.

Highlights

In spite of much effort, ligand-targeted nanoparticles have yet to achieve significant clinical impact.

The primary approach thus far to optimizing nanoparticle targeting has been largely based on empirical studies. However, fundamental principles of ligand–receptor interactions can be used to guide a more rational-design approach.

The basic principles of binding were developed *in vitro* under simple conditions with complete control over all relevant binding parameters. Adapting these for *in vivo* drug delivery requires understanding the specific challenges present *in vivo*.

Nanoparticle delivery *in vivo* is highly dynamic and, consequently, targeting should be viewed as a kinetic competition between target site and off-target accumulation.

Site-specific retention can be maximized by focusing on the two steps that drive local retention: (i) close contact between nanoparticle and target cell; and (ii) effective ligand–receptor engagement upon contact.

Translating the Fundamentals of Receptor–Ligand Interactions

Conjugation of cell-specific ligands (including antibodies) to the surface of nanoscale drug carriers (**nanoparticles**; see Glossary) can potentially increase delivery of a therapeutic agent to a desired anatomic site, while decreasing unwanted delivery to other sites. Often described as active targeting, this nomenclature can be misinterpreted to imply that ligand-conjugated drug carriers acquire the capacity to home to the intended site of therapeutic delivery [1]. However, molecular forces that mediate ligand–receptor binding (e.g., hydrogen bonds and electrostatic interactions) only extend over 0.3–0.5 nm [2,3] and therefore cannot provide a long-distance magnetic pull to a site of therapeutic need. Instead, **ligand targeting** can improve delivery by increasing the likelihood that a nanoparticle is retained by a target cell or tissue it happens to contact.

Achieving effective nanoparticle targeting is then a question of how to optimize for maximal contact and retention. While empirical approaches are typically used in nanoparticle optimization, the rigorous formalism describing receptor–ligand interactions can potentially provide a more efficient basis for rational design. However, this formalism generally assumes ideal experimental conditions optimized for specific receptor–ligand binding with negligible nonspecific interactions. By contrast, biomedical engineers seeking to exploit receptor–ligand binding for site-specific nanoparticle delivery must contend with complex conditions that offer little control over critical binding parameters (Figure 1). Thus, the central challenge is how to adapt the principles of receptor–ligand binding from the simple *in vitro* environment to the complex conditions encountered *in vivo*.

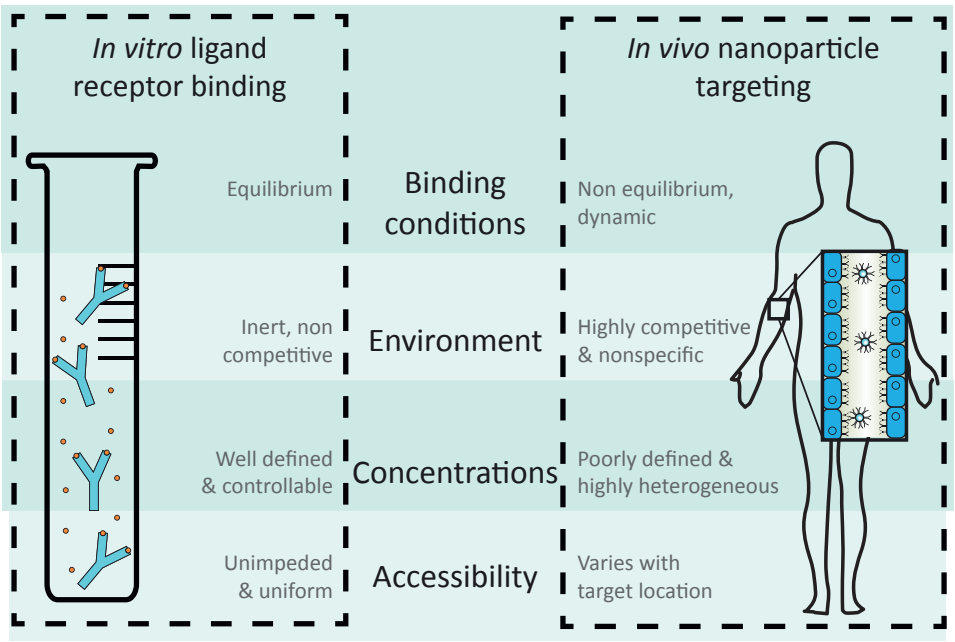
In this Opinion, we suggest an accessible framework for adapting fundamental principles of molecular binding to produce effective nanoparticle targeting *in vivo*. Because *in vivo* nanoparticle delivery is an inherently kinetic, non-**equilibrium** process, we suggest that the key to achieving effective site-specific targeting is to focus on factors that influence the rates of

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Figure 1. Key Differences in Receptor–Ligand Binding *In Vitro* versus in *In Vivo* Nanoparticle Targeting. The principles of receptor–ligand binding are often determined in simplified *in vitro* experiments, where there is negligible interaction with the inert environment, and the concentrations of ligand and receptor can be controlled to ensure equilibrium conditions are met. By contrast, systemic ligand-mediated targeting is done in a competitive environment, where the concentration of available ligands is diminished over time due to nonspecific binding and actions of the mononuclear phagocyte system. In this setting, it is unlikely that delivering ligands in excess of receptors can ever be accomplished regardless of nonspecific elimination. Additionally, even though a known amount of ligand–nanoparticle can be administered, hemodynamic effects on nanoparticle distribution within a vessel and the heterogeneity of the target receptors mean that local concentrations cannot be exactly known. Finally, the accessibility of a target *in vivo* is highly variable depending on the target cell type location. These attributes together contribute to an inherently complex, nonequilibrium environment.

nanoparticle accumulation, including rates of accumulation at both desired and undesired sites. Within this framework, we focus on two essential (and sequential) steps for effective targeted nanoparticle retention: Step I: contact between nanoparticle and the targeted cell or tissue; and Step II: successful ligand–receptor engagement (Figure 2, Key Figure). This conceptual framework can help assess which cellular targets and therapeutic settings are most likely to benefit from ligand-targeted nanomedicines. We conclude by providing an example of an optimized application: targeting vascular endothelium during *ex vivo* normothermic machine perfusion of isolated human organs being prepared for transplantation.

Winning the Kinetic Competition

Since most nanoparticles are introduced into the bloodstream, the half-life of nanoparticles in the circulation is a critical determinant of therapeutic efficacy and much effort has been expended to engineer nanoparticles with prolonged half-lives [4–6]. Half-life affects efficacy because targeted nanoparticle delivery *in vivo* is effectively a competition – for a limited supply of agent – between the desired target site versus all off-target sites. Since nanoparticle circulating concentration is continuously falling, this competition is an ever-evolving, nonequilibrium process. Consequently, the steady-state, equilibrium-based binding parameters (e.g., the equilibrium dissociation constant or $\mathbf{K_D}$) typically used to describe receptor–ligand

Glossary

CD31: or platelet–endothelial cell adhesion molecule 1 (PECAM-1), is a cell–cell adhesion protein found at the intercellular junctions of endothelial cells (as well as on the surface of platelets, monocytes, and some lymphocytes). It acts as a mechanosensor of fluid shear stress and is involved in the regulation of leukocyte migration through venular walls.

CD47: or integrin-associated protein, is a transmembrane protein ubiquitously expressed on human cells, especially circulating and immune cells, and delivers a 'don't eat me' signal to macrophages.

Equilibrium: the state where the forward reaction rate (ligand + receptor → ligand bound to receptor, or A + B → AB) is equal to the reverse reaction rate (bound ligand and receptor unbind to form free ligand and receptor). While routinely achievable *in vitro*, the equilibrium state is not generally reached during targeting of nanoparticles to cells of interest.

Intercellular cell adhesion molecule 1 ICAM1: or CD54, is a transmembrane cell adhesion protein on endothelial and other cells. Expression levels of ICAM1 dramatically increase on activated endothelial cells at sites of inflammation and it participates in arrest and transmigration of leukocytes.

K_D: equilibrium disassociation constant is equal to the ratio of the reverse rate of the reaction (bound → free receptor and ligand) over the forward rate of reaction (ligand + receptor → bound, or A + B → AB). The lower the K_D the stronger the interaction, K_D can be used to represent affinity.

Kinetic competition: the competitive environment encountered by ligand-coated nanoparticles target cells or tissues versus nonspecific elimination as by hepatic and splenic phagocytes available for binding at the target site as a function of time.

Ligand targeting: an approach that uses conjugation of ligand to the surface of a nanoparticle in order to engage specific receptors expressed on cells and tissues of interest. Ligands used for targeting may take

Key Figure

Applying the Law of Mass Action to Optimize the Two Steps to *in vivo* Ligand-Mediated NP Targeting

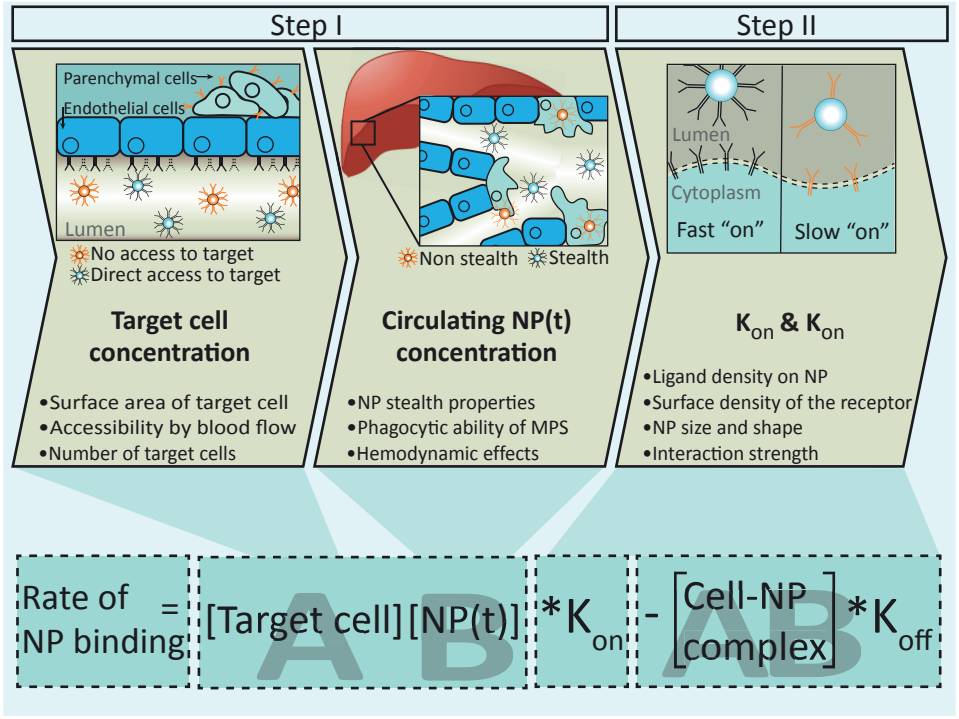


Figure 2. The first step to maximizing targeted NP retention is maximizing the frequency of NP–cell contact. In the law of mass action, which can be used to describe the rate of NP–cell binding, this term is represented with concentrations of the target cell (A) and NP (B). Targeting highly accessible cells (e.g., endothelium; left panel) versus cells in the parenchyma, allows NPs to come within close range of the target where molecular interaction forces can act, thereby allowing receptor-ligand binding to occur. Reducing NP elimination by the MPS slows the reduction of NP concentration over time, allowing for more frequent NP–cell contact (middle panel). The second step is to design successful ligand engagement of the target cell. Choosing highly expressed receptor targets and designing NP systems with high density of ligands can contribute to fast on rates and slow off rates between ligand and receptor. This will increase the likelihood that chance contact will result in retention of the NP and reduce the likelihood that the nanoparticle will be released from the cell surface prior to internalization. Abbreviations: MPS, mononuclear phagocytotic system; NP, nanoparticle.

interactions *in vitro* are less applicable *in vivo*. Instead, the efficacy of targeted delivery *in vivo* is dictated by the competitive balance between the rate of accumulation at the target site versus the combined rate of undesired accumulation at competing sites.

This **kinetic competition** has inspired delivery strategies that avoid first-pass elimination of nanoparticles from the circulation by the **mononuclear phagocyte system** [7]. Nanoparticles can be injected into the vasculature just upstream of the target organ, providing maximal particle exposure prior to subsequent phagocytic elimination. For example, intra-arterial administration of nanoparticles has been used to improve delivery to the brain in a mouse

various forms, including peptides, antibodies or antibody fragments.

Mononuclear phagocyte system: this term describes the sinusoidal macrophages of the liver and spleen that serve to remove particulate matter, including nanoparticles, from the bloodstream through phagocytosis. It replaces the earlier term, reticuloendothelial system, as the sinusoidal endothelial cells of these organs are now known to play no role in such clearance.

Nanoparticle: A submicrometer diameter drug carrier used to deliver therapeutic agents. Nanoparticles may have distinct compositions, such as lipids, crosslinked proteins, or synthetic polymers, that will carry in their capacity to bind and release drugs.

Normothermic machine perfusion: explanted organs are connected to a mechanized circuit that supplies warmed, oxygenated fluids, such as washed and suspended red blood cells, allowing restoration of function in the organ before transplantation.

Phage display libraries: bacteriophages are encoded with specific genes of interest, causing the phage to display the associated protein, interaction between the phage and molecules at a target site can help describe the surface of tissues.

model of cerebrovascular thrombosis [8,9] and to cardiac vasculature in a healthy pig model [10,11]. Since the first transit time through the target organ is short, perhaps <1 s, targeted nanoparticles must rapidly bind or extravasate to benefit from this strategy.

Not all tissue sites of therapeutic interest are amenable to immediate upstream administration, meaning phagocyte-mediated elimination of nanoparticles is often an unavoidable challenge. Consequently, targeting can also be improved by minimizing the rate of nanoparticle phagocytosis. This can be achieved through the use of inert surface coatings such as poly(ethylene) glycol (PEG) or hyperbranched poly(glycerol) that suppress phagocytic clearance [12,13]. Variation of nanoparticle size, shape, and surface charge can also modulate phagocytosis both *in vitro* and *in vivo* [4]. Biomimicry offers another possible approach. A 'don't eat me' peptide derived from human **CD47** has been conjugated to the surface of nanoparticles to reduce phagocytosis *in vivo* in NOD/SCID mice; a strain that recognizes human CD47 [14]. Alternatively, so-called cloaked or camouflaged nanoparticles are coated with the plasma membrane of platelets or red cells to impart characteristics of these cells. This has been shown to reduce phagocytic elimination in primary cell line human macrophages *in vitro* [15] and following *in vivo* delivery in mouse models [16]. Collectively, these approaches provide evidence of kinetic competition in targeted drug delivery and illustrate how to tip the balance of accumulation in favor of the intended target site.

Making Contact: The First Step in Effective Target Site Retention

Given the short-range nature of molecular interaction forces, nanoparticles must first come in close contact with the target cell before receptor–ligand binding can occur. Several factors that can influence the frequency of contact between nanoparticle and target cell have been identified [17] (Figure 2), but here we focus on the critical issue of target cell accessibility. The most prevalent mode of nanoparticle administration in both experimental models and human clinical trials is intravenous injection, and the most typical targets are solid tumor cells [18,19] (see Clinician's Corner; Table 1). However, extravascular tumor cells are poorly accessible due to the barrier formed by endothelial cells that line the vasculature and selectively regulate passage between blood and tissue. Consequently, nanoparticle targeting of tumor cells generally fails to significantly alter biodistribution *in vivo* following systemic nanoparticle administration [20,21]. While tumor vasculature is often leaky [22], this can paradoxically limit nanoparticle extravasation by increasing interstitial pressure, thereby reducing outward bulk fluid flow, the means by which nanoparticles cross the vessel wall [23]. Consequently, the fraction of administered dose that escapes the vasculature to accumulate within the tumor is generally only a minor fraction of the injected dose (~1%) [24].

In contrast to the poor accessibility of extravascular cells, vascular endothelium represents a highly accessible cell type to nanoparticles in the bloodstream. Endothelial cells perform a number of key homeostatic functions required for organ health including: (i) regulation of vascular tone; (ii) regulation of bidirectional molecule exchange between blood and tissue; (iii) active maintenance of blood fluidity by controlling coagulation; and (iv) control of inflammation by preventing adhesion and activation of innate and adaptive immune cells [25]. Injurious stimuli can interfere with the normal performance of any of these tasks, causing endothelial dysfunction, which contributes to the pathophysiology of a wide array of diseases from sepsis to cardiovascular disease to cancer [26]. In addition to homeostatic regulation, endothelial cells can be activated to perform specialized functions, such as recruitment of circulating immune cells in infection [25]. Inappropriate endothelial activation can cause inflammatory or autoimmune tissue injury. Alternatively, tumor vessel endothelium can resist activation, impairing antitumor immune responses [23]. This combination of direct accessibility and broad

pathophysiological importance in a variety of disease settings provides significant therapeutic potential for nanoparticle targeting to vascular endothelial cells.

Successful endothelial targeting of drug-loaded nanoparticles has been demonstrated *in vivo* (reviewed in [11,17,27]). Recently, a nanoparticle made of low-molecular-weight lipids, termed 7C1, was shown to specifically target endothelial cells through an unknown mechanism. These particles effectively delivered silenced nucleic acids to lung endothelium in mouse models of emphysema and Lewis lung carcinoma [28], and to aortic plaque endothelium in a mouse model of atherosclerosis [29]. Nanoparticle targeting via conjugation of endothelium-specific molecules (e.g., antibodies, natural ligands, and peptides) have also been shown to enhance site-specific targeting of endothelium. Anti-intracellular adhesion molecule **(ICAM)1** nanoparticles can increase lung targeting *in vivo* by $> 10 \times$ in a wild-type mouse model as an example [10,63]. The success of endothelial targeting underscores the importance of target cell accessibility to ensure nanoparticles frequently collide with the target cell type.

From First Contact to Stable Ligand–Nanoparticle Capture

Effective retention of a nanoparticle at an accessible site requires engagement between cell–surface–receptor and nanoparticle–surface–ligand (Figure 2). The surface density of the target receptor on the cell of interest is critically important to the efficiency of this engagement. *In vivo* **phage display libraries** have been used to generate peptides specific for receptors highly expressed on endothelium in specific tissues of interest, dubbed vascular zip codes [30–32]. Specific peptides conjugated to nanoparticles can target specific vascular beds in a variety of different mouse models of disease including: impaired uteroplacental perfusion in pregnancy [33], Alzheimer's disease [34], and abdominal aortic aneurysm [35]. Antibodies have also been routinely used to target receptors known to have high levels of expression in specific areas of interest. For example, ICAM1 has higher expression on activated endothelium at sites of

Table 1. Examples of Clinical Studies Using Targeted and Nontargeted Polymer Nanoparticles[a]

Name	Formulation	Targeting molecule	Administration route	Disease indication	Clinical phase	Refs
Abraxane	Paclitaxel–albumin	None	Intravenous	Breast cancer, NSCLC, pancreatic cancer	FDA approved	[53]
AZD2811 Nanoparticle	AZD2811–PLA-PEG	None	Intravenous	Acute myeloid leukemia/ high-risk myelodysplastic syndrome	Phase I/II	[54]
BIND-014	Docetaxel–PLA-PEG	Prostate-specific membrane antigen ligand	Intravenous	NSCLC, prostate cancer	Phase II	[55]
CALAA-01	siRNA–cyclodextrin-PEG	Transferrin	Intravenous	Solid tumors	Phase I	[56]
CRLX101	Camptothecin–cyclodextrin-PEG	None	Intravenous	Pancreatic cancer, ovarian cancer, and SCLC	Phase II	[57]
Genexol-PM	Paclitaxel–PLA-PEG	None	Intravenous	Breast, lung, pancreatic cancer	Phase III/IV	[58,59]
SEL-068	Nicotine-*t*SVP	Agonist/T cell helper peptide	Intravenous	Nicotine addiction	Phase I	[60]
SVP-rapamycin	Rapamycin–PLGA + PLA-PEG	None	Intravenous	Gout	Phase II	[61]
Yale BNPs	Avobenzone-Octocrylene–PLA-HPG	Bioadhesion to skin	Topical	Prevention of sun damage and skin cancer	Pilot	[62]

[a]Abbreviations: NSCLC, non-small cell lung cancer; PLGA, poly(lactide-co-glycolide); PLA-PEG, poly(lactic acid)-poly(ethylene glycol); PLA-HPG: poly(lactic acid)-hyperbranched polyglycerol.

inflammation relative to quiescent endothelium [36,37]. Targeting ICAM-1 has been used in a mouse model of traumatic brain injury to improve the delivery of the enzyme catalase to reduce reactive oxygen species [38]. Other molecules that are induced in endothelial cell dysfunction also have potential as target receptors. The integrin $\alpha_v\beta_3$, for example, may distinguish angiogenic tumor from normal vasculature potentially providing a mechanism to target therapeutics to these vessels [39].

Conjugate–ligand surface density is also an essential determinant of nanoparticle targeting efficacy [40,41]. Notably, studies with different particle types, sizes, and ligands have demonstrated that excessive levels of ligand density that saturate the full nanoparticle surface appear to actually impair targeting, likely due to steric hindrance [17]. In cell culture, subsaturating ligand densities were found to be optimal for three different ligands (HER2 affibody, HER2-targeting peptide, and folate) on two sizes of nanoparticles (25 and 50 nm) [41]. These steric effects, which impair the ability of the ligand to properly engage the cognate receptor, underscore the importance of the receptor–ligand binding properties. Relatedly, dual epitope targeting of **CD31** can provide collaborative enhancement by making epitopes more accessible for effective nanoparticle targeting [42]. Molecular engineering has also been used to improve molecular binding properties through the use of high-affinity, single-chain targeting antibodies [43]. Collectively, these examples demonstrate how the molecular properties of both the ligand–nanoparticle and the target cell receptor can contribute to more effective targeting.

A final determinant of the stability of nanoparticle capture and subsequent therapeutic efficacy is endocytic fate. Notably, it has been shown in primary cell culture of human umbilical vein endothelial cells that crosslinking of receptors on the cell surface by nanoparticles decorated with a high surface density of ligand can trigger endocytic internalization [44]. This process is dependent upon receptor identity, receptor crosslinking, nanoparticle size, and shape [36,45]. Moreover, the rate of internalization and the subsequent intracellular fate has also been shown to be sensitive to biological factors (e.g., shear stress, and exposure to ischemia [46,47]). Internalized nanoparticles have the potential benefit of durable retention at the target site, thereby allowing for sustained therapeutic effect by slow release of the encapsulated agent [48].

An Optimal Application: Delivery during *Ex Vivo* Organ Perfusion

The concepts reviewed in the preceding sections suggest an important conclusion: ligand-targeted nanomedicines are not suitable for every disease and therapeutic setting. Instead, just as is true for receptor–ligand binding *in vitro*, conditions must be optimal to produce the desired result. We conclude by highlighting an emerging therapeutic setting that optimizes the potential of targeted nanomedicines, namely endothelial cell targeting during *ex vivo* **normothermic machine perfusion** of isolated organ grafts. This technology aims to better preserve, assess, and revive marginal organs in order to expand the pool of organs available for clinical transplantation [49] (Figure 3). In addition, the *ex vivo* perfusion period creates a unique opportunity for direct delivery of drugs within the perfusate; an approach that can circumvent many of the challenges of systemic drug delivery. In the context of transplantation, therapeutic agents can be delivered directly to the graft endothelium to protect against ischemia reperfusion injury and reduce the intensity of immune-mediated rejection post-transplant.

We have recently demonstrated that nanoparticles administered *ex vivo* can provide prolonged siRNA-mediated knockdown of MHC molecules lasting up to 6 weeks in the endothelial cells lining a human coronary artery segment transplanted into an immunodeficient mouse [50]. We have also recently shown that conjugation of an anti-CD31 antibody can enhance vascular retention of poly(lactic acid)–PEG nanoparticles during *ex vivo* perfusion of human kidneys [51].

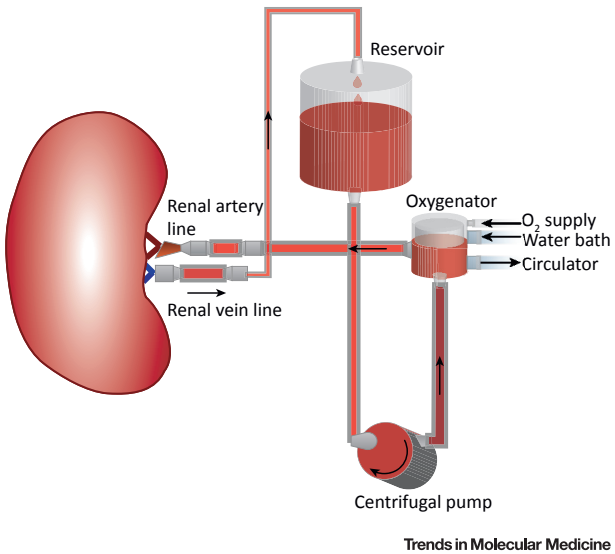


Figure 3. *Ex Vivo* Organ Perfusion as a Setting for Vascular-Targeted Nanoparticles. In this procedure, human organs (e.g., kidney) are connected to a closed circulation loop, maintained by a mechanical centrifugal pump. Perfusates, such as washed and suspended red blood cells, are kept at or near normal body temperature. Supplying continuous flow supplemented with oxygen supply and necessary nutrients extends the time that an organ can retain healthy function outside of the body while awaiting transplant. The period of *ex vivo* perfusion provides privileged access to the organ, significantly limiting the complications and variables of systemic delivery systems. The isolated nature of the organ also simplifies measurement of nanoparticle delivery characteristics and physiological function to assess any impact of therapeutic delivery.

In our study, we performed quantitative analysis of vascular-targeted nanoparticle retention in a series of eight human kidneys. This gave us an opportunity to quantitatively evaluate the efficacy of vascular targeting in an intact human organ with native 3D architecture and cellular complexity.

In addition to the potential of this work in transplantation, our recent study in human kidneys has revealed the value of *ex vivo* perfusion as a quantitative tool to assess nanoparticle targeting efficacy in a translational setting. It is becoming clear that *in vitro* cell cultures are not adequate for predicting the efficacy of targeting *in vivo* [51,52]. This is probably because, while easy to work with, cell culture does not replicate the 3D architecture and cellular complexity present *in vivo*. Isolated organs, however, can provide this complexity and may provide a bridge to overcome the challenges associated with systemic nanoparticle targeting. Furthermore, isolated organ perfusion also provides a simplified setting relative to experiments in live animals with greater control over the parameters relevant to effective targeting (e.g., perfusate composition/viscosity, and time-dependent nanoparticle concentration) and better opportunity for detailed quantitative assessment. Since these systems can be run side by side in similar experimental platforms, it is also possible that isolated organs could be used as an experimental platform to develop a rigorous formalism to predict *in vivo* drug delivery.

Concluding Remarks

Ligand-conjugated nanomedicines are not a panacea for avoiding all of the pitfalls of systemic drug administration, but this approach has the potential to be highly impactful when used in the right setting. Many questions remain (see Outstanding Questions), however, the keys to successful implementation of this technology are becoming clearer and involve careful consideration of the competitive balance of kinetics of accumulation at the target site versus all off-target sites. From this perspective, targeting vascular endothelium during perfusion of isolated organs has significant potential for rapid translational impact. However, even in an idealized setting (such as an isolated organ), realizing benefit from the addition of targeting ligands still requires careful quantitative attention to the fundamentals of ligand–receptor binding, which though undeniably more complex, are just as important *in vivo* as they are in a test tube.

Outstanding Questions

How can nanoparticle targeting efficacy be compared reliably between different research groups working at different locations? The development of a robust theoretical model to predict targeted nanoparticle accumulation will likely require the development of a parameter for easy comparison, as with the equilibrium binding affinity, or K_D, used to describe receptor–ligand interaction *in vitro*. Similarly, absolute quantitation of number of ligands per nanoparticle needs to become a standard reported parameter.

What methods can be used to quantifiably determine the absolute surface density of receptors on the target cell, as well as the total accessible surface area of the target cell within the tissue of interest?

To what extent does uptake of nanoparticles into target cells affect the rate and extent of drug release and how might targeting to different receptors influence these processes?

With respect to vascular targeting, how does the surface receptor heterogeneity vary between similar vessel types in different organs and different vessels types within the same organ?

What are the nuances of nanoparticle delivery across all length scales, from intracellular to intraorgan? There is currently little known about intraorgan biodistribution as the standard approach for analysis is to homogenize the whole organ.

Clinician's Corner

Particles with submicrometer diameter (nanoparticles, typically <200 nm) are being used as drug carriers in clinical trials in attempts to increase local drug concentration while minimizing systemic toxicity.

When administered systemically (e.g., via the bloodstream), nanoparticle-mediated delivery can be viewed as a competition between delivery to the cells of interest versus undesired phagocytic clearance by the macrophages of the liver and spleen. Some clinical trials have failed because these macrophages are highly efficient at eliminating nanoparticles leading to low levels of delivery to target cells of interest.

Attaching a ligand to the nanoparticle surface that binds to the cell or tissue of interest, referred to as targeting, can improve delivery, but only if: (i) the nanoparticles have access to the desired site (which, if extravascular, is often prevented by the endothelial cell lining of the blood vessels); and (ii) if binding of the ligand to its specific cell or tissue-associated receptor functions to efficiently capture and retain the nanoparticle at that site.

In light of these considerations, the selection of the clinical setting is very important for successful applications of targeted nanoparticle drug delivery.

As an example, endothelial cell antibodies can be used to successfully target nanoparticle drug carriers to the vascular endothelial cells of an isolated organ that is being machine perfused *ex vivo* prior to transplantation. This setting is in many ways ideal because: (i) endothelial cells, the principal cellular targets of postoperative injuries and graft rejection, are readily accessible to the bloodstream; and (ii) the isolated *ex vivo* perfusion circuit circumvents competition from hepatic and splenic macrophages in the graft recipient.

Acknowledgments

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