MINI-REVIEW

Tailoring nanoparticles for targeted drug delivery: From organ to subcellular level

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Abstract
An effective dose at specific sites is of paramount importance for disease therapy. While therapeutic reagents such as small molecule drugs, nucleic acids, peptides, and proteins are suffered from degradation or clearance by physiological environment, nanomedicine has emerged to improve drug delivery efficiency due to their superior targeting ability. By tailoring the properties of nanoparticle, including size, shape, surface chemistry, site-specific drug delivery in organ, cellular, and subcellular level could be achieved. This minireview highlights recent advances in the development of targeted drug delivery system for disease therapy.

KEYWORDS
disease therapy, nanomedicine, targeted drug delivery

1 | INTRODUCTION
Therapeutic reagents, including small molecule drugs,1 nucleic acids,2 peptides, and proteins,3 have been widely adopted in the treatment of diseases. These reagents need to reach certain organs, cells or even subcellular organelles to exert maximum effects. However, some of them suffer from short half-life during circulation, easy degradation by endogenous enzymes and immune clearance when administrated in the complex physiological environment,4 which necessitate the investigation of targeted drug delivery systems that can transport therapeutic payloads to specific sites and improve in vivo efficacy.

Nanomedicine has gained burgeoning attention in biomedical field during last few decades.5 Some nanoparticles, such as Doxil,6 CYT-6091,7 Ferumoxytol,8 targeting polymer micellar anticancer drugs (BIND-014),9 HfO2 nanoparticles (NBTXR3),10 and ThermoDox,11 are already in clinical use or in clinical trials, which have the potential to benefit numerous patients. Due to the nanoscale size, varied shape and composition, and tailorablesurface properties, nanoparticles can be rationally designed to target specific locations, providing ideal vehicles for drug delivery. To date, the researches focusing on targeted nanoparticles could be classified into following aspects according to in vivo stages of therapeutics:

1. Improving pharmacokinetics of drugs. Upon systemic administration, nanoparticulated formulations are confronted with adsorptions from serum proteins in the blood, which may alter the physicochemical properties of nanoparticles that affect their retention in biological system and targeting efficiency to specific site.12 Therefore, strategies aiming at increasing the circulation time
of drugs by shielding interferent in the blood play crucial roles in targeted therapy.

2. Avoiding entrapment in nontargeted organs and tissues. As a foreign stimulus, nanoparticle entering the body may be sequestered in the liver and spleen or eliminated by kidney, leading to reduced dose at expected target. The manipulation of interaction between nanoparticles and organs may assist in circumventing nonspecific distribution and facilitating drug accumulation in diseased site.

3. Cellular uptake. To obtain optimal therapeutic effect while avoid side effects, pharmaceuticals are entailed with the ability to distinguish healthy and diseased cells. Cargo-loaded nanoparticles may be anchored with surface ligand that can recognize particular receptor on the plasma membrane of diseased cells, preferentially releasing cargos in anticipated positions.

4. Subcellular allocation. Intracellular compartments, including nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus are tightly linked with physiologic homeostasis. Since disorders at subcellular level typically endow hallmarks of disease, targeting organelles with delicately designed nanoparticles may advance medication to a more precise stage.

In summary, transporting certain pharmaceuticals into diseased organs and tissues with minimum clearance during blood circulation, differentiating cellular uptake of nanoparticles, and priming drug delivery to a subcellular level are essential topics in the field of nanomedicine. This mini review discusses recent advances of targeted drug delivery platforms from organ to organelle level that may accomplish the goal of precision medicine (Figure 1).

2 | MATERIAL DESIGN

It has been reported that the physicochemical properties of nanoparticles, including size, shape, charge, composition, and surface chemistry will significantly affect their interaction with biological systems, thus changing their in vivo distribution. Modulation of these properties to accommodate different situations represents a promising way for targeted drug delivery.

2.1 | Size

Nanoparticles with different size are distinguished by their retention time in vivo, penetration ability across tissues and cellular uptake efficiency, which may significantly influence the therapeutic outcome. Therefore, understanding how particle size affects corresponding biological response and function is essential for the designing of therapeutic platform.

In nanomedicine-mediated anticancer strategies, nanoparticles can passively reach tumor via enhanced permeability and retention (EPR) effect of vasculature. While larger particles tend to retain in tumor tissues, their penetration into deeper places far away from blood vessel is limited. On the contrary, smaller ones own enhanced penetration depth, but they face the problem of rapid clearance by kidney. In light of this dilemma, Li et al. reported a stimuli-responsive clustered nanoparticle (iCluster) to overcome several biological barriers in solid tumors by benefiting from the size of different nanoparticles. The iCluster was prepared by assembling platinum prodrug-conjugated poly(amidoamine) dendrimers (diameter ~5 nm) into polymeric clustered nanoparticles.
(diameter ~100 nm). After accumulated at tumor site with high retention ratio, the iCluster could alter its structure by tumor extracellular acidity and release the smaller platinum prodrug, which greatly facilitated the tumor penetration and cell internalization of drugs.

2.2 | Shape

In addition to particle size, the shape of nanoparticles will also influence the cellular uptake and intracellular delivery of therapeutics, thus adjusting targeting ability toward certain positions. Liu et al. synthesized four types of uniform nanostructures, including spheres, large compound vesicles, smooth disks, and unprecedented staggered lamellae with spiked periphery via PEG-b-PCPTM polyprodrug amphiphiles made from poly(ethylene glycol) and camptothecin (CPT) prodrug monomer. They explored shape-dependent blood circulation duration, cellular uptake, internalization pathway, as well as drug release profile, in vitro particle biodegradation and cytotoxicity, which highlighted the shape-tunable biological performance of nanoparticles derived from polyprodrug amphiphiles and may inspire more studies related to the geometry-controlled cellular targeting.

2.3 | Surface modification

Besides the “passive targeting” based on modulating physiochemical properties of nanoparticles, “active targeting” by surface modification with specific ligands that can recognize receptors on targeted cells is another promising way to increase targeting efficiency. Proteins, peptides, polysaccharides, aptamers, and small molecules are widely adopted ligands for nanoparticle-mediated drug delivery.

Kim et al. developed an oligopeptide complex consisting of adipocyte-targeting sequence and 9-arginine (ATS-9R) to selectively deliver a short-hairpin RNA (shRNA) for silencing fatty-acid-binding protein 4 (shFABP4) in adipocytes, which effectively reduced the body weight of obese mice.

Ma et al. found that nanoscale polysaccharides-based glucose polymers could target adipose macrophage in obese mice. They prepared a series of dextran conjugates linked to different imaging agents or anti-inflammatory drugs, which modulated the phenotype of macrophage in visceral adipose tissue, resulting in a significant reduction of pro-inflammatory markers in obese mice.

Our group also established several tumor specific platforms for efficient targeting. By utilizing tumor cell-penetrating peptide (PEGA-pVEC) and polysaccharide hyaluronic acid (HA) as targeting media, chemotherapeutic drug doxorubicin (Dox) and drug resistance-related small interfering RNA (siRNA) were specifically delivered into breast tumor in vivo after being encapsulated in a rattle mesoporous silica scaffold. Besides, cascaded aptamers that targeting the HER2 receptor upregulated on the surface of tumor cell and high intracellular ATP content were organized into a envelope-type nanovehicle to precisely release anticancer drug in HER2-overexpressing breast cancer cells.

Small-molecule ligands also have multiple applications in specific targeting. Biotin and triphenylphosphonium cation, were selected by Tang et al. to target biotin receptor on the surface of tumor cells and intracellular mitochondria, respectively. The dual-targeted organic molecules were then conjugated with photothermal agent to kill tumor cells under near-infrared light, which exhibited excellent tumor inhibitory effects.

In addition to above-mentioned properties, other parameters, such as surface charge and hydrophilicity also provide enter points to engineer nanoparticles for amplified targeting ability, which greatly diversify the strategies of site-specific drug delivery.

3 | TARGETED REGION

Since key parameters in material design were summarized in last section to achieve enhanced targeting efficiency, specific region related with certain disease is the subsequent issue that should be taken into account. The targeted site where drugs exert their functions depends on the therapeutic mechanism of that drug. However, restricted by highly organized architecture in vivo, the designed nanomaterials face great challenges to overcome various physiological barriers to reach targeted region. This section mainly focuses on nanoparticle-mediated drug delivery in organ, cellular, and subcellular level.

3.1 | Organ

It is reported that most nanoparticles accumulate at liver and will be eliminated from the body through hepatobiliary pathway after interacting with hepatocytes, resulting in lowered dose at lesion and elevated liver toxicity. Therefore, exploiting organ-specific drug delivery system beyond the liver will elicit more favorable therapeutic outcome.

Recently, Siegwart et al. reported a selective organ targeting (SORT) nanoparticle for tissue-specific mRNA delivery and CRISPR-Cas gene editing. On the basis of traditional lipid nanoparticles, addition of a supplementary SORT molecule precisely manipulated the in vivo delivering profile of mRNA, rendering lung-, spleen-, and liver-specific accumulation, respectively. More importantly,
the proposed methodology is well-suited in various gene editing substances, including mRNA, Cas9 mRNA/single guide RNA and Cas9 ribonucleoprotein complexes, which may potentiate the application of protein replacement and gene correction therapeutics with accurately designed nanoparticles (Figure 2A).

Choi et al.\textsuperscript{31} engineered a series of PEGlyated gold-based nanoparticles with different size ($\text{Au}_x\cdot\text{PEG}_y$) to target kidney. They investigated the blood pharmacokinetics, organ-level, and cellular-level distribution of synthesized nanoparticles and determined that the optimal one with a diameter of $\sim 75 \pm 25$-nm preferentially accumulated at kidney mesangium, providing a constructive principle for the engineering of kidney-targeting therapeutics.

Besides, abnormally deposited extracellular matrix is also found to be a therapeutic target in certain diseases like tumor,\textsuperscript{32,33} which acts as an alternative candidate beyond certain tissues.

### 3.2 Cell

Cellular identification and discrimination are of fundamental significance in disease therapy owing to prevalent pathogenesis at cellular level. Nanoparticles have been employed to target diverse types of cell due to their tailororable properties, which provide controllable and tunable carriers for cell specific cargo delivery.
Instead of targeting tumor cells, the strategy of targeting tumor-associated fibroblasts (TAFs) to treat desmoplastic tumors was employed by Huang et al.\textsuperscript{39} Plasmids encoding the secretable TNF-related apoptosis-inducing ligand (sTRAIL) were first encapsulated into lipid-based nanoparticles. Contrary to traditional direct targeting approach, off-target distribution of nanoparticles from tumor to fibroblasts was selected to generate sTRAIL-producing TAFs. By targeting the expression of secreted cytotoxic proteins from TAFs, in vivo tumor growth inhibition was achieved in an orthotopic xenograft model of human pancreatic.

Besides fibroblasts, systemic delivery of vaccine antigens into dendritic cells (DCs) represents an expedient but challenging method in cancer immunotherapy.\textsuperscript{35} By solely modulating net charge of lipid carriers without ligand functionalization, intravenously injected RNA-lipoplexes (RNA-LPX) could be efficiently delivered into dendritic cells, as reported by Kranz and Diken et al.\textsuperscript{36} The RNA-LPX could precisely promote DC maturation to induce inflammatory immune response, which hold great promise for systemic nanoparticulate vaccine.

Anderson et al. engineered a lipid nanoparticle (LNP) system to deliver messenger RNA to B lymphocytes in spleen.\textsuperscript{37} Cholesterol, lipid-anchored (PEG), and phospholipid were formulated into ionizable lipid materials by microfluidic mixing, which was denoted as OF-Deg-Lin. Together with mRNA encapsulation, the OF-Deg-Lin could induce over 60 pg of protein expression per million B cells inside the spleen (Figure 2B), which demonstrated an effective vector for mRNA delivery into B lymphocytes.

Dysfunctional endothelium also causes a variety of diseases.\textsuperscript{38} To achieve effective gene editing in endothelial cells for disease therapy, Sago et al.\textsuperscript{39} developed a high-throughput in vivo screen system named FIND to identify nanoparticles for the delivery of functional mRNA. A series of lipid nanoparticles (LNPs) were prepared to deliver Cre mRNA and a unique DNA barcode. Then the identification process began with the screen of Cre gene-edited cells by FACS after administration to Cre reporter cells or mice, followed by the sequence of DNA barcode to distinguish LNPs that delivered the mRNA. Based on this platform, simultaneous quantification of more than 100 lipid nanoparticles could be achieve to obtain well-matched target cells.

3.3 Subcellular compartment

Subcellular compartments, including nucleus, lysosome, mitochondria, endoplasmic reticulum, and Golgi apparatus, are closely associated with many fundamental life-sustaining activities, such as DNA replication, self-renewal, cellular metabolism, and protein synthesis.\textsuperscript{40} Dysfunction of these intracellular organelles demands targeted drug delivery at subcellular level.

Many chemotherapeutics such as doxorubicin, an anticancer drug that can cause DNA damage in nucleus, need to arrive at nucleus to elicit therapeutic effect.\textsuperscript{41} Typically, nanoparticles can enter into the nucleus through size-dependent passive diffusion mediated by the open channel of the nuclear pore complex\textsuperscript{42} or nuclear localization signals (NLS)-dependent active transport.\textsuperscript{43} To investigate the role of ligand density and particle size in mediating nuclear targeted delivery, quantum dots with different size and NLS density were selected to characterize the intracellular trafficking of nanoparticles.\textsuperscript{44} It was illustrated that the smallest particle (3.0 nm) exhibited the highest nucleus localization among three investigated sizes (3.0, 4.8, 8.0 nm) and 20% surface NLS density presented to be the plateau for nuclear targeting within 30 minutes. These findings provided feasible methods to engineer nanoparticles to achieve best nucleus targeting.

Lysosome is responsible for the digestion of many types of biological substances and functions as a hub for the transit of various nanoparticles. Current application of lysosome targeting relies on its acidic environment and degradative enzymes.\textsuperscript{45} For example, Hu et al.\textsuperscript{46} prepared a lysosome-targeting nanoparticle based on the bis-styryl BODIPY dyes. The designed nanoparticles could absorb near-infrared (NIR) light and experience acid-activable photodynamic therapy as well as photoacoustic imaging, which provided a new platform for the cancer theranostics.

Mitochondria is the main organelle for cellular energy production and metabolism maintenance, and is therefore an important target in the treatment of cancer.\textsuperscript{47} Triphenylphosphonium (TPP) cation,\textsuperscript{48} heterocyclic aromatic cations,\textsuperscript{49} and specific peptides\textsuperscript{50} are mainstream moieties for mitochondria targeting. Cheng et al.\textsuperscript{51} reported a dual-targeted chimeric peptide that aimed at plasma membrane and mitochondria. After being conjugated with a hydrophobic photosensitizer protoporphyrin IX (PpIX) and self-assembled into spherical micelles (designated as M-ChiP), the peptide could realize the synergistic plasma membrane and mitochondria targeting and enhance the photodynamic therapy-induced cell apoptosis.

Correlated with calcium equilibrium, protein folding, and lipid synthesis, endoplasmic reticulum (ER) and Golgi apparatus (GA) maintain the biological homeostasis.\textsuperscript{16} While elevated ER stress and GA abnormality supported the tumorigenesis and metastasis, targeting ER-Golgi network may block the pathway of metastatic cancer.\textsuperscript{52} Celecoxib (CLX) inhibiting upregulated cyclooxygenase 2 at GA\textsuperscript{53} and protein
transport inhibitor brefeldin A (BFA) were coencapsulated into the PLGA nanoparticles (CBNPs). CLX impeded COX-2 induced cell migration, vascular permeability, and upregulated matrix metalloproteinase-9 (MMP-9) or vascular endothelial growth factor (VEGF) expression. At the same time, BFA facilitated the fusion between GA and ER, increasing ER stress that resulted in cancer cell apoptosis (Figure 2C). Enhanced cytotoxicity and metastasis prevention could be observed in murine metastatic breast cancer cells.

4 | SUMMARY AND OUTLOOK

We have summarized recent advances of targeted drug delivery systems based on engineered nanoparticles. Although remarkable achievements have been made, several challenges still exist that need to be appropriately addressed. First, the basic mechanism of nano-bio interaction still remains to be elucidated to provide guidelines for the design of targeted platforms, which requires more fundamental research in this field. In some cases, targeting efficiency cannot be significantly increased to a satisfactory level despite of complicated and elaborated configurations, emphasizing the importance of seeking more potent targeting moiety, and avoiding overdesign of therapeutics. The uncertain biosafety of nanomaterials is a detrimental restriction that limits their clinical translation, which should be underlined in biomedical applications. Safe, effective, and simple nanoparticles combined with other drugs for multimechanism therapy or diagnosis and treatment may be the main direction of nanomedicine development. We believe that targeted drug delivery systems based on nanoparticulated formulations would gain substantial progress in tackling above-mentioned issues and benefit more patients in the near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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