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Review

# Extra- and intra-cellular fate of nanocarriers under dynamic interactions with biology

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ABSTRACT

Synthetic nanocarriers continue to play an important role in nanomedicine as the next generation of therapeutics demands effective intracellular delivery. Better understanding of the extra- and intra-cellular fate of the nanocarriers in relation with biological environments *in vitro* and *in vivo* is essential for the progress of nanocarrier-based therapeutics. As nanocarriers come into contact with biological fluid, interactions with various biomolecules alter their initial physicochemical status. Such dynamic modifications influence their interactions with cells and the subsequent endocytic process. Upon cellular uptake, intracellular machineries control their trafficking, release and degradation of cargoes, and exocytosis. In this Review, we highlight the navigation of nanocarriers through various extra- and intra-cellular barriers, with an emphasis on how dynamic interactions with biological components alter their fate and performance.



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## Introduction

With the development of nanotechnology, clinical therapeutics has faced an innovative paradigm of maximizing therapeutic potential with reduced harmful side effects. Nanocarriers are biomedical materials developed to host and deliver therapeutic and diagnostic molecules, ranging from small drugs, peptides, proteins, to genetic molecules. In fact, a broad spectrum of compositional choices (polymeric, metallic, and inorganic materials, and their combinations/hybrids), and various morphologies (nanospheres, nanorods, nanoshells, nanoplates, nanofibers, etc) have been substantially sought out. Furthermore, methodologies to modify the nanocarrier chemistry have also been piled up to improve the loading efficiency and delivery capacity. Thus far, the therapeutic and diagnostic efficacies of the developed nanocarriers have primarily been demonstrated in many in vitro models. Additionally, some *in vivo* studies utilizing the nanocarriers have been promised to circumvent many biological obstacles that otherwise are not easily amenable to gain therapeutic body functions.

Despite such research developments in the nanocarriers, studies on their extra- or intra-cellular fate have relatively been less explored. The dynamic molecular and cellular interactions of the nanocarriers during their long journey along the extra- and intra-cellular roads should ultimately determine their carrier performance and the biological fate. As overviewed in Fig. 1, nanocarriers travel around the in vitro or in vivo extracellular environment, and then meet the cell to enter into the intracellular compartment, a process called 'endocytosis'. Endocytosis involving multiple stages begins to engulf nanocarriers in the membrane invaginations pinched off to form vesicles, known as endosomes. Nanocarriers in endosomes are delivered to their different destinations by undergoing various intracellular processes or the recycle process termed 'exocytosis'.

The current Review aims to zoom up the extra- and intra-cellular events of the nanocarriers, highlighting their interactions with biological molecules, cellular uptake processes, intracellular trafficking and the recycling pathways. This is hoped to sum up and improve our understanding on the nanocarrier fate in biological interactions, and ultimately to explore nanocarriers with improved performance in effective loading of the cargo molecules and their targeted and controlled delivery to wanted sites.

## Nanocarriers and guest molecules

Since nanocarriers are in principle developed to load guest molecules, the properties of nanocarrier entities are important in the interactions with biology. In this section, the loading principle of guest molecules onto nanocarriers is briefly discussed and the possible effects on the biological journey of nanocarriers are addressed.

Thus far, a number of methods have been explored to effectively load the guest molecules, such as chemical drugs, proteins, peptides, and nucleic acids, within nanocarriers. There are roughly two approaches for constructing nanoparticle entities; covalent and non-covalent interactions between host and guest (Table 1).

Covalent conjugates of host-guest have shown several significant advantages. Firstly, water-soluble polymer conjugation enables to dramatically improve the water-solubility and bioavailability of hydrophobic molecules, e.g., PEGylation [9–11]. Secondly, delivery of drugs conjugated with nanocarriers can be achieved in a controlled manner. In this way, the release pattern may be customized by the conjugation degree, potentially diminishing the fluctuation of periodic administration. As a result, it is possible to avoid undesired side effects, toxicity, and damage of organs or tissues [12,13]. Thirdly, host-guest conjugation often renders labile or chemically-reactive molecules to be stabilized, preventing severe toxicity of drugs associated with burst leaching out. Thus, the covalent conjugation of host-guest can prolong the circulation time in blood and control the release profile of drug [14,15]. For example, water-soluble yet highly toxic cisplatin derivatives were covalently conjugated with nanocarriers to stabilize loading and control the release with significantly reduced toxicity [16]. However, this covalent approach is in many cases time-consuming compared to the non-covalent approach.

In contrast, an approach of using non-covalent host-guest interactions is a more efficient and simpler way of formulating nanoparticle entities [17]. This approach includes i) hydrogen bonding, ii) electrostatic charge–charge interaction, iii) hydrophobic interaction, and iv) physical adsorption. Compared to the covalent approach which depends on the conjugation yield of host-guest, non-covalent approaches have generally higher loading capacity. In an example, doxorubicin loading quantity is improved by hydrogen bonding of the carbonyl group of drug molecules to the urea group in the polymeric micelle core [18]. Similarly, acid-base interactions between drug and micelle core contribute to high drug loading capacity [19,20]. Charge-charge interactions also provide high loading capacity of charged molecules such as nucleic acids and proteins by tailoring the carrier surface [20–22]. Particularly for negatively-charged genetic molecules, positively-charged carriers with proven biocompatibility are used, which include polyethylenimine (PEI), chitosan, polyamidoamine (PAMAM) dendrimers, and poly(L-lysin) (PLL). These positively-charged polymers are often hybridized with inorganic and metallic nanocarriers for multi-functionality, e.g., additionally providing diagnostic ability. For example, chlorotoxin-labeled PEI-coated iron oxide nanoparticulate system was designed for glioma cell-targeting and magnetic resonance imaging [23]. Unlike the ionized molecules, hydrophobic drugs are generally encapsulated by means of chemical precipitation and disintegration processes, such as high-pressure homogenization and media milling to enhance the systemic bioavailability and drug absorption. In some cases, stabilizers or surfactants are also added at large quantities. For example, injectable paclitaxel and docetaxel were formulated with polyethoxylated castor oil (Cremophor® EL) and polysorbate 80 (Tween 80®), respectively [24]. However, these additives can evoke allergic reactions, hypersensitivity, neurotoxicity, and nyelosuppression [25]. Recently, advanced chemical procedures involving hydrophobic interactions between drug and host enabled these hydrophobic drugs to be encapsulated with enhanced systemic bioavailability and effective drug absorption. For example, block copolymer micelles composed of polycaprolactone (PCL) branched to poly(ethylene glycol) (PEG) allowed for efficient encapsulation of hydrophobic docetaxel, and consequently significantly increased in the systemic bioavailability [26]. The physical adsorption of drugs to carriers has been a simple and effective way to improve the drug loading capacity. In this case, increasing the adsorption site is a proper way to increase the loading efficiency. A hollow and porous structure of nanoparticles is able to aid in loading hydrophobic drugs due to the high surface area [27,28]. For example, hollow structured superparamagnetic iron oxide nanoshell presented an effective encapsulation of hydrophobic drugs with a high stability in aqueous media [29].

## Interactions of nanocarriers with biological fluid and proteins

Upon the entry into a biological fluid, nanocarrier entities encounter various biological ingredients and thereby undergo degradation and/or meet reticuloendothelial system (RES). Thus,

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