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Multifunctional Pharmaceutical Nanocarriers

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Preface

The use of various pharmaceutical carriers to enhance the in vivo efficiency of many drugs and drug administration protocols has been well established during the last decade in both pharmaceutical research and clinical setting. Surface modification of pharmaceutical nanocarriers, such as liposome, micelles, nanocapsules, polymeric nanoparticles, solid lipid particles, and niosomes, is normally used to control their biological properties in a desirable fashion and to simultaneously make them perform various therapeutically or diagnostically important functions. The most important results of such modification include an increased stability and half-life of drug carriers in the circulation, required biodistribution, passive or active targeting into the required pathological zone, responsiveness to local physiological stimuli, and ability to serve as contrast agents for various imaging modalities (gamma-scintigraphy, magnetic resonance imaging, computed tomography, ultra-sonography). Frequent surface modifiers (used separately or simultaneously) include soluble synthetic polymers (to achieve carrier longevity); specific ligands, such as antibodies, peptides, folate, transferrin, and sugar moieties (to achieve targeting effect); pH- or temperature-sensitive lipids or polymers (to impart stimuli sensitivity); chelating compounds, such as EDTA, DTPA, and deferoxamine (to add a heavy metal-based diagnostic/contrast moiety onto a drug carrier).

Certainly, new or modified pharmaceutical carriers (nanocarriers) as well as their use for the delivery of various drugs and genes are still described in many publications. However, looking into the future of the whole field of drug delivery, we have to think about the development of the next generation of pharmaceutical nanocarriers, combining variety of properties and allowing for the simultaneous performance of multiple functions. The current level of engineering pharmaceutical carriers in some cases allows for drug delivery systems, demonstrating a combination of several desired properties. Long-circulating immunoliposomes represent a good example of this approach as they combine the ability to remain in the circulation for a long time with the ability to specifically accumulate in target areas. One may add pH-sensitive long-circulating liposomes and micelles, or nanocarriers simultaneously loaded with a drug and an imaging agent, to the list. Such nanocarriers belong to the new, “smart” generation of drug delivery systems. In principle, we can

imagine drug delivery systems, which, depending on the immediate requirements, can simultaneously or sequentially demonstrate the following properties: (1) circulate long in the blood or, more generally, stay long in the body; (2) specifically target the site of the disease (accumulate there) via both nonspecific and/or specific mechanisms, such as enhanced permeability and retention (EPR) effect and ligand-mediated recognition; (3) respond local stimuli characteristic of the pathological site, such as intrinsic abnormal pH values or temperature or externally applied heat, magnetic field, and ultrasound, by, for example, releasing an entrapped drug or deleting a protective coating and facilitating the contact between drug-loaded nanocarriers and target pathological cells; (4) provide an enhanced intracellular delivery of an entrapped drug in case the drug is expected to exert its action inside the cell (gene delivery to the nuclei or delivery of proapoptotic drugs to the mitochondria surface are good examples); (5) supply real-time information about the carrier (and drug) biodistribution and target accumulation as well as the outcome of the therapy due to the presence within the structure of the carrier of a certain reporter/contrast group. Some other less significant and more exotic functions can also be "attached." Strictly speaking, the term "multifunctionality" may also be applicable to pharmaceutical carriers simultaneously loaded with more than one drug type. To meet the requirements listed above, drug carrier should simultaneously carry various moieties capable of functioning in a certain orchestrated and coordinated fashion. Thus, for example, if a system that can provide the combination of longevity (allowing for the target accumulation via the EPR effect) and specific cell binding (allowing for its internalization by target cells) has to be constructed, two requirements have to be met. First, the half-life of the carrier in the circulation should be long to fit EPR effect requirements. Second, the internalization of the carrier within the target cells should proceed fast to avoid carrier degradation and drug loss in the interstitial space. We have to agree that systems like this still represent a challenge, although a certain work in this direction has already been done and certain examples of multifunctional matrices for oral and tumoral delivery already exist.

This book attempts to cover an emerging area of multifunctional pharmaceutical carriers. It includes 15 chapters describing different aspects of this approach, from stimuli-responsive long-circulating micelles to magnetically sensitive drug carriers, which can be simultaneously used as imaging agent. Certainly, a single book cannot include all the currently available information, and the potential reader may discover that certain areas of interest are absent in this volume. Still, I feel that it is a good beginning.

I am deeply grateful to all my friends and colleagues who have contributed to this book. As an editor, I am open to comments and advices from our readers and I believe that they will find this book useful.

Boston, MA Vladimir Torchilin

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Multifunctional Pharmaceutical Nanocarriers: Development of the Concept

Vladimir P. Torchilin

1 Multifunctionality of Pharmaceutical Carriers: What to Expect?

The use of nanoparticulate pharmaceutical carriers to enhance the in vivo efficiency of many drugs and drug administration protocols well established itself over the past decade both in pharmaceutical research and in clinical setting. Certainly, new or modified nanocarriers as well as their combinations with various drugs and genes are still described in multiple publications. However, looking into the future of the field of drug delivery, we have to think about the development of the next generation of pharmaceutical nanocarriers combining variety of properties and allowing for the simultaneous performance of multiple functions. Considering the task within our current level of understanding on what is good in drug delivery systems (DDSs), we can imagine a DDS, which, depending on the immediate requirements, can simultaneously or sequentially demonstrate the following properties: (1) circulate long in the blood or, more generally, stay long in the body; (2) specifically target the site of the disease (accumulate there) via both non-specific and specific mechanisms, such as enhanced permeability and retention (EPR) effect and ligand-mediated recognition; (3) respond to the local stimuli characteristic of the pathological site, such as intrinsic abnormal pH values or temperature or externally applied heat, magnetic field or ultrasound, by, for example, releasing an entrapped drug or deleting a protective coating and thus facilitating the contact between drug-loaded nanocarriers and cancer cells; (4) provide an enhanced intracellular delivery of an entrapped drug in case the drug is expected to exert its action inside the cell (gene delivery to the nuclei or delivery of proapoptotic drugs to the mitochondria surface); (5) supply a real-time information about the carrier (and drug) biodistribution and target accumulation as well as about the outcome of the therapy due to the presence of a certain reporter or contrast moiety within the structure of the carrier. Some other, less significant and more exotic functions can also be "attached." Strictly speaking, the term *multifunctionality* may also be applicable to pharmaceutical carriers simultaneously loaded with more than one drug type, but such systems have not be discussed here. Any way, to be able to meet the requirement listed earlier, a drug carrier should simultaneously carry on its surface various moieties capable of

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functioning in a certain orchestrated order. The schematic structure of such pharmaceutical carriers is shown in Fig. 1. We have to agree that systems like this still represent a challenge, although a certain work in this direction is already done and certain examples of multifunctional matrices for oral and tumoral delivery have even been already reviewed (Bernkop-Schnurch and Walker, 2001; Torchilin, 2006a; van Vlerken and Amiji, 2006).

Various pharmaceutical nanocarriers, such as nanospheres, nanocapsules, liposomes, micelles, cell ghosts and lipoproteins, are widely used for experimental (and already clinical) delivery of therapeutic and diagnostic agents (Domb et al., 2007; Thassu et al., 2007; Torchilin, 2006b). Surface modification of these carriers is often used to control their properties in a desirable fashion and make them to

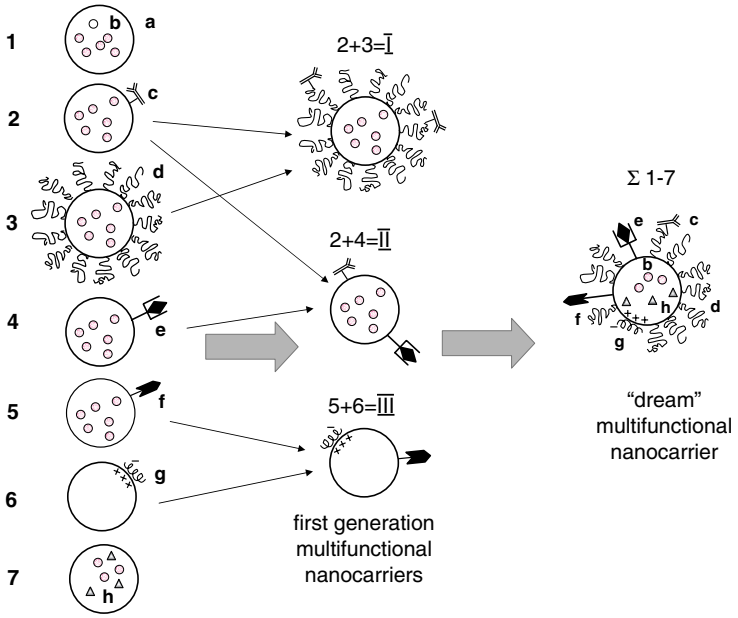

Fig. 1 Typical representatives of monofunctional pharmaceutical nanocarriers: (1) traditional "plain" nanocarrier (a, nanocarrier; b, drug loaded into the carrier); (2) targeted nanocarrier or immunocarrier (c, specific targeting ligand, usually a monoclonal antibody, attached to the carrier surface); (3) long-circulating nanocarrier [d, surface-attached protecting polymer (usually PEG) allowing for prolonged circulation of the nanocarrier in the blood]; (4) contrast nanocarier for imaging purposes (e, heavy metal atom – ^{111}In, ^{99m}Tc, Gd, Mn – loaded onto the nanocarrier via the carrier-incorporated chelating moiety for γ- or MR imaging application); (5) cell-penetrating nanocarrier (f, cell-penetrating peptide, CPP, attached to the carrier surface and allowing for the carrier enhanced uptake by the cells); (6) DNA-carrying nanocarrier such as lipoplex or polyplex (g, DNA complexed by the carrier via the carrier surface positive charge); (7) magnetic nanocarrier (h, magnetic particles loaded into the carrier together with the drug and allowing for the carrier sensitivity towards the external magnetic field and its use as a contrast agent for magnetic resonance imaging). First-generation multifunctional pharmaceutical nanocarriers may include different combinations of individual functions – see examples I, II and III. Hypothetical "dream" multifunctional pharmaceutical nanocarrier combines the properties of all monofunctional carriers 1–7

simultaneously perform several different functions. The most important results of such modification(s) include increased longevity and stability of the carrier (and carrier-incorporated drug) in the circulation, favorably changed biodistribution, targeting effect, stimuli (pH or temperature)-sensitivity and contrast properties. Frequent surface modifiers (used separately or simulatenously) include soluble synthetic polymers (to achieve carrier longevity); specific ligands, such as antibodies, peptides, folate, transferrin and sugar moieties (to achieve targeting effect); pH- or temperature-sensitive lipids or polymers (to impart stimuli-sensitivity); chelating compounds, such as EDTA, diethylene triamine penta acetic acid (DTPA) or deferoxamine (to add a heavy-metal-based diagnostic or contrast moiety onto a drug carrier). Evidently, different modifiers can present on the surface of the same nanoparticular drug carrier in different combinations, providing it with a set of useful properties required in each particular case (e.g. longevity and targetability, targetability and stimuli-sensitivity, or longevity, targetability and contrast properties). See the general scheme in Fig. 1.

Chemical or physical conjugation of proteins, peptides, polymers and other molecules to the carrier surface required to produce multifunctional pharmaceutical nanocarriers with controlled properties can proceed covalently or non-covalently, via the hydrophobic adsorption of certain intrinsic or specially inserted hydrophobic groups in the ligands. Thus, amphiphilic polymers or hydrophobically modified proteins can adsorb on the hydrophobic surface of polystyrene nanoparticles (Yuan et al., 1995) or incorporate into the phospholipid membrane of liposomes (Torchilin, 1998) or hydrophobic core of micelles (Torchilin, 2001). The attachment can also be performed chemically, via the reaction of reactive groups generated on the carrier surface and certain groups in the molecule to be attached. In many cases, the conjugation methodology is based on three efficient and selective reactions: reaction between activated carboxyl groups and amino groups yielding an amide bond, reaction between pyridyldithiols and thiols yielding disulfide bonds and reaction between maleimide derivatives and thiols yielding thioether bonds (Torchilin and Klibanov, 1993). Some other approaches also exist, for example yielding the carbamate bond via the reaction of the *p*-nitropheylcarbonyl groups introduced onto the surface of nanocarriers with amino group of various ligands (Torchilin et al., 2001b). The detailed review of numerous coupling procedures and protocols used for attaching the whole variety of surface modifiers to drug carriers can be found in Klibanov et al. (2003) and Torchilin et al. (2003c).

It was shown, for example, that carboxylic groups of immunoglobulins can be activated by water-soluble carbodiimide; activated protein then can be bound to free amino-group-containing surfaces, such as phosphatidyl ethanolamine (PE)-containing liposomes (Dunnick et al., 1975). For further ligand attachment, corresponding reactive groups on the surface of nanocarriers can be pre-modified with the aid of heterobifunctional cross-linking reagents, such as popular *N*-succinimidyl-3 (2-pyridyldithio)propionate (SPDP) reagent, which was used for the synthesis of a PE derivative further used for the coupling to SH-containing proteins (Leserman et al., 1980). Another possibility is to rely on the reaction of the thiol groups on a ligand (protein) with the maleimide-group-carrying surfaces. This approach (Martin

and Papahadjopoulos, 1982) is now one of the most widely used in research and practical applications. For example, various high and low molecular weight compounds have been attached to liposomes, the most popular drug carrier, by using pyridyldithiopropionyl-PE or maleimide reagents (Klibanov et al., 2003; Torchilin et al., 2003c). The most interesting is the application of free thiol groups on immuno globulin Fab fragments. The main advantages of this procedure are its simplicity and the possibility of controlling the progress of the reaction.

Some ligands carry carbohydrate residues, which can be easily oxidized to yield aldehyde groups that can react with surface aminogroups, for example with liposomal aminophospholipids (e.g. PE), with the formation of the Schiff bases (Heath et al., 1980). Liposomes containing carboxyl-bearing derivatives of PE were used for the attachment of different ligands (Kung and Redemann, 1986) after the activation with water-soluble carbodiimide directly prior to ligand addition. Same chemical reactions can be used to attach non-modified proteins and peptides to various nanocarriers, including pre-formed liposomes, containing membrane-incorporated reactive lipid derivatives, such as *N*-glutaryl-PE or glutaryl-cardiolipin (Bogdanov et al., 1988; Weissig and Gregoriadis, 1992; Weissig et al., 1990). The use of a four-tailed hydrophobic cardiolipin derivative instead of a two-tailed PE derivative allows for a decrease in the number of amino groups involved in the conjugation reaction at the same degree of hydrophobicity. This results in better activity preservation by the hydrophobized and liposome-attached protein (Niedermann et al., 1991; Weissig et al., 1986). Some methods for attaching various ligands to nanocarriers are reviewed in Nobs et al. (2004).

2 Longevity of Pharmaceutical Nanocarriers in the Blood

For the body defence system, "plain" pharmaceutical nanocarriers usually represent foreign particles. As a result, they become easily opsonised and eliminated from the circulation long before the completion of their function. Thus, the longevity function of pharmaceutical nanocarriers becomes prerequisite, and long-circulating pharmaceuticals and pharmaceutical carriers represent currently an important and still growing area of biomedical research (Cohen and Bernstein, 1996; Lasic and Martin, 1995; Moghimi and Szebeni, 2003; Torchilin, 1996, 1998; Torchilin and Trubetskoy, 1995b). The longevity of drug carriers allows maintaining a required level of a pharmaceutical agent in the blood for extended time intervals. In addition, long-circulating drug-containing microparticulates or large macromolecular aggregates can slowly accumulate [EPR effect, also termed as *passive* targeting or accumulation via an impaired filtration mechanism; see Maeda (2001) and Maeda et al. (2000)] in pathological sites with compromised and leaky vasculature (such as tumors, inflammations and infarcted areas), and facilitate drug delivery in those areas (Gabizon, 1995; Maeda, 2001; Maeda et al., 2000). In addition, the prolonged circulation can help to achieve a better targeting effect for targeted (specific ligand-modified) drugs and drug carriers, allowing more time for their interaction with the target (Torchilin, 1996).

The most frequent way to impart the in vivo longevity to drug carriers is their chemical modification with certain synthetic polymers, such as poly(ethylene glycol) (PEG), as was first suggested for liposomes (Allen et al., 1991; Klibanov et al., 1990; Maruyama et al., 1991; Papahadjopoulos et al., 1991; Senior et al., 1991). Hydrophilic polymers have been shown to protect individual molecules and solid particulates from interaction with different solutes providing what is named *steric stabilization* (Napper, 1983). Coating nanoparticles with PEG sterically hinders interactions of blood components with their surface and reduces the binding of plasma proteins with nanoparticles as was demonstrated for liposomes (Allen, 1994; Chonn et al., 1991, 1992; Lasic et al., 1991; Senior et al., 1991; Woodle, 1993), thus preventing drug carrier interaction with opsonins and their fast capture by RES (Senior, 1987) due to the formation of the polymeric layer over the particle surface, which is impermeable for other solutes even at relatively low polymer concentrations (Gabizon and Papahadjopoulos, 1992; Torchilin et al., 1994). Currently, there exist many chemical approaches to synthesize activated derivatives of PEG and to couple these derivatives with a variety of drugs and drug carriers [see reviews in Torchilin (2002), Veronese (2001) and Zalipsky (1995)]. Thus, for example, to make PEG capable of incorporating into the liposomal membrane, the reactive derivative of hydrophilic PEG is single terminus modified with hydrophobic moiety (usually, the residue of PE or long-chain fatty acid is attached to PEG-hydroxysuccinimide ester) (Klibanov et al., 1990, 1991). In majority of protocols, PEG-PE is used, which must be added to the lipid mixture prior to liposome formation. Alternatively, it was suggested to synthesize single end-reactive derivatives of PEG able to be coupled with certain reactive groups (such as maleimide) on the surface of already prepared liposomes, referred to as the post-coating method (Maruyama et al., 1995). Spontaneous incorporation of PEG-lipid conjugates into the liposome membrane from PEG-lipid micelles was also shown to be very effective and did not disturb the vesicles (Sou et al., 2000).

Although PEG is the golden standard in making long-circulating drugs and drug carriers, quite a few other biocompatible, soluble, and hydrophilic polymers have also been suggested as alternative steric protectors for nanoparticular drug carriers (Torchilin and Trubetskoy, 1995b; Torchilin et al., 1995b), such as single terminus lipid-modified poly(acryl amide) and poly(vinyl pyrrolidone) (Chonn et al., 1992; Lasic et al., 1991), poly(acryloyl morpholine) (Monfardini et al., 1995; Ranucci et al., 1994; Sartore et al., 1994), phospholipid (PE)-modified poly(2-methyl-2-oxazoline) or poly(2-ethyl-2-oxazoline) (Woodle et al., 1994), phosphatidyl polyglycerols (Maruyama et al., 1994) and polyvinyl alcohol (Takeuchi et al., 1999).

Surface modification of hydrophobic polymeric nanoparticles can be performed by physical adsorption of a protecting polymer on a particle surface, or by chemical grafting of polymer chains onto a particle. Possible examples of the first case include the absorption of series of polyethylene oxide and polypropylene oxide copolymers (Pluronic/Tetronic™ or Poloxamer/Poloxamine™ surfactants) on the surface of polystyrene latex particles via the hydrophobic interaction mechanism, and resulting polymer-coated nanoparticles also become protected from the uptake by reticuloendothelial system upon intravenous injection (Illum and Davis, 1983). The absorption

of the above copolymers leads not only to the decrease of particle uptake by resident macrophages in liver but, after coating with some specific copolymers, can redirect the injected nanoparticles to other organs (Porter et al., 1992). For example, the coating of 60-nm polystyrene latex with Poloxamer 407 results in increased particle accumulation in bone marrow. The same group has demonstrated that analogous procedure also helps substantially to alter the biodistribution of subcutaneously injected nanospheres. Coating of 60-nm diameter polystyrene nanospheres with certain Poloxamer/Poloxamine copolymers results in their increased accumulation in regional lymph nodes. The optimal length of the copolymer polyoxyethylene block for this particular purpose has been found to be 5–15 oxyethylene units. Non-coated particles normally stay at the injection site while particles coated with longer polyoxyethylene-containing copolymers are not retaining in the nodes and eventually appearing in systemic circulation (Moghimi et al., 1994). Surface modification of polystyrene latexes with PEG was also successfully applied to make long-circulating particles and study their penetration into tumors (Hobbs et al., 1998; Monsky et al., 1999; Yuan et al., 1995).

Another important type of polymeric nanoparticles is based on the block-copolymer of PEG and polylactide-glycolide (PEG-PLAGA) (Gref et al., 1994, 1995; Krause et al., 1985). Using PLAGA-PEG copolymer, one can prepare long-circulating particles with insoluble (solid) PLAGA core and water-soluble PEG shell covalently linked to the core (Gref et al., 1994, 1995). Similar effects on longevity and biodistribution of microparticular drug carriers might be achieved by direct chemical attachment of protective polyethylene oxide chains onto the surface of preformed particles (Harper et al., 1991). Similarly, coating polycyanoacrylate particles with PEG resulted in their increased longevity in the circulation, allowing even for their diffusion into the brain tissue (Calvo et al., 2001; Peracchia et al., 1999). Fluorouracil-containing dendrimer nanoparticles modified with PEG demonstrated better drug retention and less hemolytic activity (Bhadra et al., 2003). Grafting PEG onto the surface of gold particles via mercaptosilanes expectedly resulted in decreased protein adsorption onto modified particles and less platelet adhesion (Zhang et al., 2001).

Thus, the most significant biological consequence of nanocarrier modification with protecting polymers is a sharp increase in its circulation time and decrease in their RES (liver) accumulation (Klibanov et al., 1990; Torchilin, 1998; Torchilin et al., 1994). This fact is very important clinically, since various long-circulating nanocarriers have been shown to effectively accumulate in many tumors via the EPR effect (Gabizon and Papahadjopoulos, 1988; Gabizon, 1995; Maeda, 2001; Maeda et al., 2000). Long-circulating liposomes were prepared containing various anticancer agents, such as doxorubicine, arabinofuranosylcytosine, adriamycin, and vincristin (Allen et al., 1992; Boman et al., 1994; Gabizon et al., 1994; Huang et al., 1994). PEG-liposome-incorporated doxorubicine (Doxil®) has already demonstrated very good clinical results (Ewer et al., 2004; Gabizon, 1995; Rose, 2005). From a pharmacokinetic point of view, the presence of protective polymer on the carrier surface further improves the parameters favorably influenced by drug association with nanocarriers, such as delayed drug absorption, restricted drug