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Introduction. Nanocarriers for Drug Delivery: Needs and Requirements

Vladimir Torchilin

Fast developing nanotechnology, among other areas, is expected to have a dramatic impact on medicine. The application of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems has recently been determined by the NIH as nanomedicine. Among the approaches for exploiting nanotechnology developments in medicine, various nanoparticulates offer some unique advantages as pharmaceutical delivery systems and image enhancement agents.^{1,2} Several varieties of nanoparticles are available³: different polymeric and metal nanoparticles, liposomes, micelles, quantum dots, dendrimers, microcapsules, cells, cell ghosts, lipoproteins, and many different nanoassemblies. All of these nanoparticles can play a major role in diagnosis and therapy. This book is attempting to present the broad overview of different nanoparticulate drug delivery systems with all their advantages and limitations, as well as potential areas of their clinical applications.

The paradigm of using nanoparticulate pharmaceutical carriers to enhance the *in vivo* efficiency of many drugs, anti-cancer drugs, first of all, well established itself over the past decade both in pharmaceutical research and clinical setting, and does not need any additional proofs. Numerous nanoparticle-based drug delivery and drug targeting systems are currently developed or under development.^{4,5} Their use aims to minimize drug degradation upon administration, prevent undesirable side effects, and increase drug bioavailability and the fraction of the drug accumulated in the pathological area. Pharmaceutical drug carriers, especially the

ones for parenteral administration, are expected to be easy and reasonably cheap to prepare, biodegradable, have small particle size, possess high loading capacity, demonstrate prolonged circulation, and, ideally, specifically or non-specifically accumulate in required pathological sites in the body.⁶

High molecular weight (40 kDa or higher), long-circulating macromolecules, including proteins and peptides, conjugated with water-soluble polymers, are capable of spontaneous accumulations in various pathological sites such as solid tumors, infarcts, and inflammations via the enhanced permeability and retention effect (EPR).^{7,8} This effect is based on the fact that pathological (tumor, infarct) vasculature, unlike vasculature of healthy tissues, is "leaky", i.e. penetrable for macromolecules and nanoparticles which allows for macromolecules to accumulate in the pathological tissue (such as interstitial tumor space). In the case of tumors, such accumulation is also facilitated by the fact that lymphatic system, responsible for the drainage of macromolecules from normal tissues, is virtually not working in case of many tumors as the result of the disease.⁸ It has been found that the effective pore size of most peripheral human tumors range from 200 nm to 600 nm in diameter, with a mean of about 400 nm. The EPR effect allows for passive targeting to tumors and other pathological sites based on the cut-off size of the leaky vasculature.⁹

Among particulate drug carriers, liposomes, micelles and polymeric nanoparticles are the most extensively studied and possess the most suitable characteristics for encapsulation of many drugs and diagnostic (imaging) agents. Many other systems meeting certain more specific requirements (and reviewed in this book) are also suggested and currently under development. Making these nanocarriers multifunctional and stimuli-responsive can dramatically enhance the efficiency of various drugs carried by these carriers. These functionalities are expected to provide: (a) prolonged circulation in the blood^{10,11} and the ability to accumulate in various pathological areas (such as solid tumors) via the EPR effect (protective polymeric coating with PEG is used for this purpose)^{12,13}; (b) ability to specifically recognize and bind target tissues or cells via the surface-attached specific ligand (monoclonal antibodies as well as their Fab fragments and some other molecules are used for this purpose)¹⁴; (c) ability to respond local stimuli characteristic of the pathological site by, for example, releasing an entrapped drug or specifically acting on cellular membranes under the abnormal pH or temperature in disease sites (this property could be provided by surface-attached pH- or temperature-sensitive coatings); (d) ability to penetrate inside cells bypassing the lysosomal degradation for efficient targeting of intracellular drug targets (for this purpose, the surface of nanocarriers may be decorated by cell-penetrating peptides). Those are just the most evident examples. Some other specific properties can also be listed, such as an attachment of diagnostic moieties. Even the use of individual functionalities is already associated with highly

positive clinical outcome — the success of Doxil®, doxorubicin in long-circulating PEG-coated liposome, represents a good example.¹⁵

In addition, there are numerous engineered constructs, assemblies, architectures, and particulate systems, whose unifying feature is the nanometer scale size range (from a few to 250 nm). Together with already listed systems, these include cyclodextrins, niosomes, emulsion particles, solid lipid particles, drug nanocrystals, metal and ceramic nanoparticles, protein cage architectures, viral-derived capsid nanoparticles, polyplexes, cochleates, and microbubbles.^{4,5,16–19} Therapeutic and diagnostic agents can be encapsulated, covalently attached, or adsorbed on to such nanocarriers. These approaches can easily overcome drug solubility issues, particularly with the view that large proportions of new drug candidates emerging from high-throughput drug screening initiatives are water insoluble. Yet, some carriers have a low capacity to incorporate active compounds (e.g. dendrimers, whose size is in the order of 5–10 nm). There are alternative nanoscale approaches for solubilization of water insoluble drugs too.^{20–23} One approach is to mill the substance and then stabilize smaller particles with a coating; this forms nanocrystals in size ranges suitable for oral delivery, as well as for intravenous injection.^{24,25} Pharmacokinetic profiles of injectable nanocrystals may vary from rapidly soluble to slowly dissolving in the blood.

In general, the development of drug nanocarriers for poorly soluble pharmaceuticals represents a special task and still faces some unresolved issues. The therapeutic application of hydrophobic, poorly water-soluble agents is associated with some serious problems, since low water-solubility results in poor absorption and low bioavailability.²⁶ In addition, drug aggregation upon intravenous administration of poorly soluble drugs might lead to such complications as embolism²⁷ and local toxicity.²⁸ On the other hand, the hydrophobicity and low solubility in water appear to be intrinsic properties of many drugs,²⁹ since it helps a drug molecule to penetrate a cell membrane and reach important intracellular targets.^{30,31} To overcome the poor solubility of certain drugs, clinically acceptable organic solvents are used in their formulations,²⁸ as well as liposomes³² and cyclodextrins.¹⁶ Another alternative is associated with the use of various micelle-forming surfactants in formulations of insoluble drugs.

By virtue of their small size and by functionalizing their surface with synthetic polymers and appropriate ligands, nanoparticulate carriers can be targeted to specific cells and locations within the body after intravenous and subcutaneous routes of injection. Such approaches may enhance detection sensitivity in medical imaging, improve therapeutic effectiveness, and decrease side effects. Some of the carriers can be engineered in such a way that they can be activated by changes in the environmental pH, chemical stimuli, by the application of a rapidly oscillating magnetic field, or by application of an external heat source.^{19,33–35} Such modifications

offer control over particle integrity, drug delivery rates, and the location of drug release, for example, within specific organelles. Some are being designed with the focus on multifunctionality; these carriers target cell receptors and deliver drugs and biological sensors simultaneously. Some include the incorporation of one or more nanosystems within other carriers, as in the micellar encapsulation of quantum dots; this delineates their inherent nonspecific adsorption and aggregation in biological environments.³⁶

The use of nanoparticulate drug carriers seems to be especially important for developing efficient anticancer therapies. Although significant advances have occurred in our understanding of tumor origin, growth, metastasis, and many different types of pharmacological agents have been developed over the years to treat tumors, the problem of optimum delivery remains a formidable challenge. For any of the drug therapy strategies to be effective, the agent must be able to reach the tumor mass in sufficient concentration, traverse through the tumor microcirculation, diffuse into the interstitium, and remain at the site for the duration to induce tumoricidal effect. As was already mentioned, due to the porosity of the tumor vasculature and the lack of lymphatic drainage, blood-borne macromolecules and nanoparticles are preferentially distributed in the tumor via the EPR effect. However, nanoparticles can also be actively targeted to tumors by modifying their surface with certain cell-specific ligands for receptor-mediated uptake. The use of specific "vector" molecules can further enhance tumor targeting of nanocarriers or make them the EPR-effect independent. The latter is especially important for the cases of tumors with immature vasculature, such as tumors in the early stages of their development, and for delocalized tumors. Vector molecules (those having affinity toward ligands characteristic for target tissues), capable of recognizing tumors were found among antibodies, peptides, lectins, saccharides, hormones, transferrin and some low molecular weight compounds (riboflavin, folate). From this list, antibodies and their fragments provide the most universal opportunity to target various for targeting and have the highest potential specificity. Vector molecules can be used for the targeting of nanoreservoir delivery systems as well. PEG-modified long-circulating doxorubicin-containing immunoliposomes targeted with anti-HER-2/neu monoclonal antibody fragments represent a recent example of increased efficiency of targeted delivery systems.³⁷ In all studied HER2-overexpressing models, immunoliposomes showed potent anticancer activity superior to that of control non-targeted liposomes. In part, this superior activity was attributed to the ability of the immunoliposomes to deliver their load inside the target cells *via* the receptor-mediated endocytosis, which is obviously important if the drug's site of action sites locates inside the cell.

An important problem is associated with the clearance of drug carriers from the circulation. Nanoparticulate pharmaceutical carriers administered into the systemic

circulation will be essentially removed within an hour of administration by the macrophages of the reticulo-endothelial system. To prolong the circulation of nanoparticles by evading the macrophages, their surface is modified with water-soluble polymers. Poly(ethylene glycol) (PEG) is very popular for surface modification of nanoparticulate drug delivery systems, since it has a long history of safe usage in biological and pharmaceutical products. Surface-bound PEG chains extend into the aqueous physiological environment and repel proteins, decrease antibody formation, and increase the circulation of the formulation in the plasma for extended period of time by the steric repulsion mechanism.³⁸

With rapid advances in molecular biology and genetic engineering, there is an unprecedented opportunity for delivery of drugs and genes to intracellular targets.³⁹ In the case of cancer, for instance, the effectiveness of many anticancer drugs is limited due to its inability to reach the target site in sufficient concentrations and to exert the pharmacological effect. Current gene delivery systems are classified as being either viral or non-viral in origin. Viruses are efficient in delivery of genes; however, they suffer from poor safety profile. Non-viral gene delivery systems, albeit not as efficient as viruses, have promise of safety and reproducibility in manufacturing. To enhance delivery of drugs to intracellular targets and gene transfection efficiency using non-viral delivery systems, it is necessary to identify ways of overcoming the cellular barriers, for example, by using various cell-penetrating proteins and peptides.^{40,41}

Self-assembled nanosystems (nanoassemblies) for targeting subcellular organelles, such as the mitochondria, are also developed.⁴² It has become increasingly evident that mitochondrial dysfunction contributes to a variety of human disorders. Moreover, since the middle of 1990s, mitochondria, the “power houses” of the cell, have also become accepted as the cell’s “arsenals”, which reflects their increasingly acknowledged key role during apoptosis. Based on these recent developments in mitochondrial research, increased pharmacological and pharmaceutical efforts have led to the emergence of “Mitochondrial Medicine” as a whole new field of biomedical research.

Nanoparticulate drug delivery systems are very important for the delivery of peptide and protein drugs and may represent a valid alternative to soluble polymeric carriers used earlier. The use of this type of carriers allows achieving much higher active moiety/carrier material ratio compared with “direct” molecular conjugates. They also provide better protection of protein and peptide drugs against enzymatic degradation and other destructive factors upon parenteral administration, because the carrier wall completely isolates drug molecules from the environment. All nanoparticulate carriers have the size, which excludes a possibility of renal filtration. Among particulate drug carriers, liposomes are the most extensively studied and poses the most suitable characteristics for protein (peptide) encapsulation.

Similar to macromolecules, protein and peptide drug-bearing liposomes are capable of accumulating in tumors of various origins *via* the EPR effect.^{6–8} In some cases, however, the liposome size is too large to provide an efficient accumulation via the EPR effect presumably due to relatively small tumor vasculature cut off size.^{43,44} In such cases, alternative delivery systems with smaller sizes, such as micelles (prepared, for example, from PEG-phospholipid conjugates) can be used. These particles lack the internal aqueous space and are smaller than liposomes. Protein or peptide pharmaceutical agent can be covalently attached to the surface of these particles or incorporated into them via chemically attached hydrophobic group (“anchor”).

In conclusion, even a brief listing of some key problems of nanocarrier-mediated drug delivery shows how broad and intense this area is. In addition to this, nanoscale-based delivery strategies are beginning to make a significant impact on global pharmaceutical planning and marketing. The leading experts in the area of nanoparticulate-mediated drug delivery attempted to address these and many other topics in this book. We strongly believe that every reader will find the book useful and stimulating.

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