

Review

Anticancer Drug Delivery with Nanoparticles

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Abstract. Nanotechnology provides a variety of nanoscale tools for medicine. Among them nanoparticles are revolutionizing the field of drug delivery. These drug nanocarriers have the potential to enhance the therapeutic efficacy of a drug, since they can be engineered to modulate the release and the stability and to prolong the circulation time of a drug, protecting it from elimination by phagocytic cells or premature degradation. Moreover, nanoscale carriers can be tailored to accumulate in tumour cells and tissues, due to enhanced permeability and a retention effect or by active targeting using ligands designed to recognize tumour-associated antigens. Could these nanomedicine tools mark an end to the necessity for loco-regional drug delivery?

Nanotechnology is the development and engineering of devices so small that they are measured on a molecular scale (Figure 1).

Nanotechnology is being applied to almost every field of scientific research, including electronics, magnetics, optics, information technology, materials development and biomedicine. Nanomedicine is a large area of application, where devices such as nanoparticles, nanomachines, nanofibers and optical and mechanical nanosensors (1, 2) could bring fundamental benefits (3).

Nanoscale drug devices are currently being developed to deliver anticancer therapeutics specifically to tumors. Nanoparticles and liposomes are the "first generation" of these devices. Some of them have already reached the clinical practice, such as liposomal doxorubicin used to treat specific forms of cancer, or liposomal amphotericin B used to treat

fungal infections often associated with aggressive anticancer treatment (4). Recently, a nanoparticulate formulation of the well-known anticancer compound taxol was submitted as a new treatment for advanced stage breast cancer (5).

Commonly, nanoparticles will target certain tissues strictly because of their size and/or their physico-chemical properties; but new types of intelligent nanoparticles that respond to an externally applied field, be magnetic, focused heat, or light, in ways that might make them ideal therapeutics or therapeutic delivery vehicles, are under examination. For example, iron oxide nanoparticles, which can serve as the foundation for targeted magnetic resonance imaging (MRI) contrast agents, can be heated to temperatures lethal to a cancer cell merely by increasing the magnetic field at the very location where they are bound to tumor cells.

Great interest in nanoparticles is due also to the fact that they are the base for the construction of *multifunctional nanoscale devices*, an issue that will not be covered in this paper, but that is under great attention in the research community. These multifunctional nanoscale devices would offer the opportunity to utilize new approaches to therapy, such as to combine a diagnostic or imaging agent with a therapeutic agent and even a reporter of therapeutic efficacy in the same package. They could be derived virtually with each new targeting ligand discovered by modern proteomics, be a monoclonal antibody, an Fv fragment to a tumor surface molecule, a ligand for a tumor-associated receptor, or another tumor-specific marker. Nanoparticles are the basis also for creating *cellular nanofactories*, capable of synthesizing and secreting multiple therapeutic compounds (6-8).

In this paper, the state of the art in nanoparticles drug delivery of anticancer agents is presented. Despite the astounding potential of these devices, it appears that much work is still needed to overcome common *drug delivery and toxicity issues* (9). Possibly, the combination of *loco-regional delivery* with nanoparticles could be a helpful strategy, in many cases.

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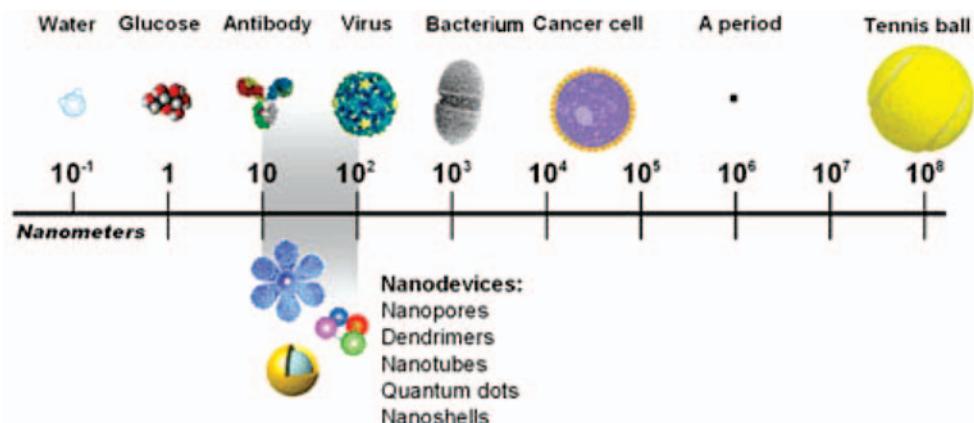


Figure 1. The scale of nanotechnology. Nanotechnology devices are characterized by dimensions in the sub-cellular (or macromolecular) range.

Nanoparticles for Drug Delivery

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). Materials at the nanometer scale often have different physical and biochemical properties from those of the same materials at bulk volume – properties that make nanostructures attractive for diagnostic and therapy applications. Since the size of the nanoparticles is significantly smaller than a cell, they can deliver a large payload of drugs, contrast agents or fluorescent probe onto the surface or interior of the cell, without disrupting its function (10-13).

These particles are able to deep penetrate tissues, going through the fenestration of the small blood-vessel epithelial tissue. They can enter the systemic blood circulation without forming blood platelet aggregates. Their reduced particle size entails high surface area and hence a strategy for faster drug release. Drug delivery rates and particle integrity can be modulated and controlled by engineering carriers in such a way that they can be activated by changes in the environmental pH (14), chemical stimuli (15) by the application of a rapidly oscillating magnetic field, or by application of an external heat source (16).

Therapeutic and diagnostic agents can be encapsulated, covalently attached, or adsorbed into such nanocarriers while the nanoparticle surface can be functionalized with synthetic polymers and appropriate ligands. Such techniques enable researchers to modulate the pharmacokinetic profiles of injectable nanocrystals which may vary from rapidly soluble in the blood to slowly dissolving, making the drug release system controllable.

Among the engineered constructs investigated and developed for this specific target are: polymeric micelles, dendrimers, polymeric and ceramic nanoparticles, protein cage architectures, viral-derived capsid nanoparticles,

polyplexes and liposomes (17). Examples of their uses are shown in Table I.

There are several techniques for producing *polymeric nanocarriers*, such as soft lithography, nanoimprinting and injection molding, which are capable of fabricating nanostructures with complicated patterns and other easier processing methods for producing *polymer membranes* with nanopores, nanofibers, nanotubes and multiple nanofilms/layers (32).

A variety of polymeric nanoparticle applications as drug delivery systems, have been presented in the literature. For instance, Lu *et al.* (33) have developed a polymeric drug delivery system for paclitaxel, synthesizing poly-lactic acid-nanoparticles by ultrasonic emulsification and demonstrated how this system inhibits the growth of ovarian carcinoma xenografts.

Natural polymers can also be used to manufacture nanocarriers for drug delivery. Among them the most utilized polymers are gelatin, dextran and chitosan. In general these nanoparticles have high encapsulation efficiency.

Abraxis Oncology (Los Angeles, CA, USA) produces ABRAXANE® Injectable Suspension, which is an injectable suspension of paclitaxel *protein-bound nanoparticles* (albumin-bound) (31, 34). The advantage of using a protein-based carrier is that albumin normally transports nutrients to cells and has been shown to accumulate in rapidly growing tumors. ABRAZANE® is solvent-free, so solvent-related toxicities are eliminated, which enables higher doses of paclitaxel to be administered.

Gold nanoparticles represent a novel technology in the field of particle-based tumor-targeted drug delivery. Paciotti *et al.* (19) have reported an application of these carriers for the targeted delivery of tumor necrosis factor-alfa (TNF- α) to solid tumors.

Quantum dots have the potential to dramatically improve clinical diagnostic tests for the early detection of cancer. These

Table I. Examples of the uses of nanoparticles for diagnostic and therapeutic applications.

Nanoparticles	Characteristics	Applications	Ref
Gold nanoparticles	Are prepared easily, have low toxicity and can be attached to molecules of biological interest. The laser light used to visualize the particles is a wavelength that causes only minimal damage to biological tissues.	This technology might enable tracking of a single molecule of a drug in a cell or other biological samples. As a vector for tumor directed drug delivery.	(18) (19)
Quantum dots (QDs)	Nanoscale crystals of semiconductor material that glow or fluoresce when excited by a light source such as a laser.	QDs can be used for high-throughput cell-based studies with the advantage of multi-plexing (<i>i.e.</i> , multiple leads tested simultaneously). Adsorption and aggregation of QDs in biological environments.	(20)
Dendrimers	3-D nanoscale core-shell structures. Polyvalent dendrimers interact simultaneously with multiple drug targets.	Conjugated to different biofunctional moieties, such as folic acid, using cDNA oligonucleotides to produce clustered molecules, which target cancer cells that overexpress the high affinity folate receptor. Linked with multiple types of molecules, which bind selectively to cancer cells, which fluorescent or used as a chemotherapy agent. Conjugated with antibodies that act as recognition sites to kill cancer cells. Targeted delivery of small molecular drugs, proteins/peptides and genes.	(21) (22) (23)
Nanobodies	The smallest available intact antigen-binding fragments harbouring the full antigen-binding capacity of the naturally occurring heavy-chain antibodies.	Potential of a new generation of antibody-based therapeutics in addition to diagnostics for diseases such as cancer.	(24)
Lipoparticles	Enable integral membrane proteins to be solubilized but retain their intact structural conformation, which is essential during assay development.	Optimal lead selection and optimization. To improve selective drug delivery by targeting tumor vasculature. As a potential carrier to deliver a lipophilic antitumor drug into hepatoma cells. Passive tumor targeting, vaccine adjuvants, gene delivery, targeting to cell surface ligands in various organs/areas of pathology, <i>etc.</i>	I.M.Inc (5) (25)
Magneto-fluorescent nanoparticles	Magnetic and fluorescent.	<i>In vivo</i> imaging rapid screening. Thermotherapy. Locoregional delivery of chemotherapeutic agents in cancer treatment.	(26) (27) (28)
Polymeric nanoparticles	Are prepared easily, have no/low toxicity, polymers can be degradable or non-degradable, synthetic or natural.	Targeted treatment of cancer. Delivery by avoiding the reticuloendothelial system, antibody-targeted therapies, targeting delivery through angiogenesis.	(25) (29) (30)
Ceramic nanoparticles	These materials can be synthesized readily at ambient temperatures with the desired size, shape and porosity.	Drug delivery system for photodynamic cancer therapy.	(31)

engineered semiconductor particles combine cadmium with selenide in a tightly packed atomic structure that emits light in a spectrum of six colours, plus four near-infrared colours, as the dots decrease in size. By finely tuning the size of the dots, thousands of subtle colour variations could be created. These tiny glowing particles, when conjugated with anti-bodies,

peptides, proteins, or DNA, form bioconjugated dots that can act as markers on cells and genes, giving scientists the ability to rapidly and differentially mark pathologic tissues.

Dendrimer-based drug delivery molecules have several potential advantages: dendrimers are comparable in size to proteins, being small enough (<5.0 nm in diameter) to escape

the vasculature and target tumor cells, while also being below the threshold of renal filtration to allow urinary excretion. For instance, acetylated dendrimers have been conjugated to folic acid, methotrexate, tritium, fluorescein and 6-carboxytetramethylrhodamine, in order to allow simultaneous treatment and drug uptake monitoring in tumors (22).

Lipoproteins are another interesting type of vector for lipophilic drugs that can be incorporated into the apolar core without affecting lipoprotein recognition. They could be recognized and taken up *via* specific receptors and mediate cellular uptake of the carried drug. In addition, they are biodegradable. Although only low density lipoproteins have been explored intensively as drug carriers for cancer chemotherapy, new investigations are focused on the use of high density lipoproteins (HDL). Bin Lou *et al.* (25) have shown that a recombinant complex of HDL and aclacinomycin, prepared by co-sonication, is able to deliver a drug to hepatoma cells.

Magnetic-drug targeting can offer a unique opportunity to treat malignant tumors loco-regionally. Alexiou *et al.* (28) have treated squamous cell carcinoma *in vivo* with the injection of magnetic nanoparticles (ferrofluids) bound to mitoxantrone, as a chemotherapeutic agent, that was locally induced to concentrate by means of a magnetic field. The intra-tumoral accumulation of the particles can additionally be visualized by means of MRI.

Finally, ceramic-based nanoparticles have been extensively investigated because of their enormous potential in the photodynamic cancer therapy (PCT) field. This is an emerging modality for the treatment of a variety of oncological, cardiovascular, dermatological and ophthalmic diseases. PCT is based on the concept that light-sensitive species or photosensitizers can be preferentially localized in tumor tissues upon systemic administration. Roy *et al.* (31) have shown that ultra-fine organically modified silica-based nanoparticles, carrying a water-insoluble photosensitizing anticancer drug-dye, 2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), were efficiently taken up by tumor cells *in vitro*, and light irradiation of such impregnated cells resulted in significant cell death.

Drug Delivery and Toxicity Related Issues

Diffusion of macromolecules, as well as that of nanoparticles, is a critical issue in drug delivery. The diffusion of microscopic objects through tissues is a multifactorial process, depending on tissue type, anatomical location, extracellular matrix composition and many other parameters. Distribution, organization and relative levels of collagen, decorin and hyaluronan, for instance, are known to impair the diffusion of macromolecules and nanoparticles in tumors (35).

Blood perfusion heterogeneities additionally impair nanoparticle diffusion in solid tumors, resulting in heterogeneous and unpredictable distribution. As

demonstrated by Jain, structural and functional abnormalities of blood and lymphatic vessels can impair an efficient delivery of macromolecules, as well as that of nanoparticles, within solid tumors (36).

Although nanoparticles are ideal tools to modulate and overcome the solubility and stability issues of drug administration, questions have been raised regarding their toxicity. In fact, over the past couple of years, a number of toxicology reports have demonstrated that exposure to certain nanotechnology derived particles pose serious risks to biological systems (37). For instance, exposure of human keratinocytes to insoluble single-wall carbon nanotubes has been associated with oxidative stress and apoptosis (38).

Common toxicity issues have often been ignored in this exciting field of research. For instance, what is the ultimate fate of nanocarriers and their constituents in the body, particularly those which are not bio-degradable, such as functionalized carbon nanotubes and coating agents, such as poly(ethyleneglycol)? Can polymeric vectors used for gene delivery, as well as other polymer-based biomaterials, interfere with cellular machineries or induce altered gene expression? What are the long-term consequences? To what extent can we translate cellular and immunological toxicity results observed in animal models to humans?

The issue of toxicity becomes particularly serious for intravenously injected nanoparticles, as the systemic circulation greatly enhances the probability of improper targeting. Loco-regional delivery could be a rational way of administration of nanoparticles, when intravenous administration is considered dangerous. But what about local accumulation of non-biodegradable objects?

Conclusion

Due to the tremendous potential of nanotechnology, science is significantly investing in this field. The National Institute of Health (NIH) Roadmap's 'Nanomedicine Initiatives' envisage that nanoscale technologies will begin yielding more medical benefits within the next 10 years (39).

In the meantime, scientists should not forget to properly address issues related to the interaction of nanomaterial with biological systems and possible safety problems, if successful and efficient application of these technologies is going to be achieved. The future of nanomedicine will depend on rational design of nanotechnology materials and tools based around a detailed and thorough understanding of the biological processes rather than the forcing applications of some materials currently in vogue.

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