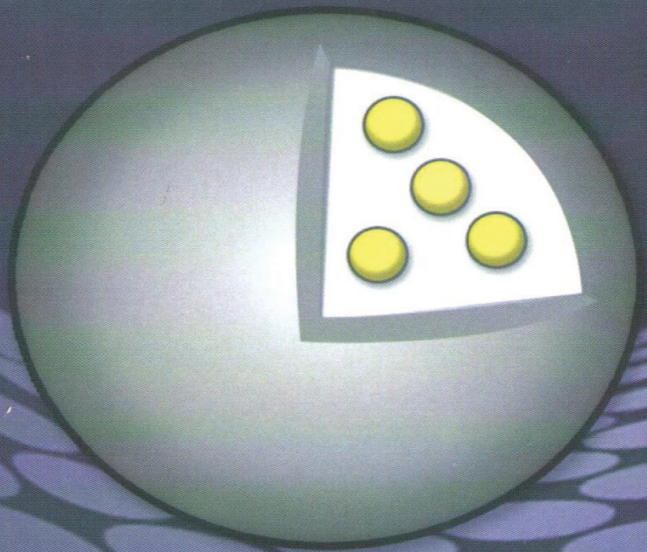
Nanomedicine for Drug Delivery and Therapeutics



Edited by

Ajay Kumar Mishra

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The book provides a comprehensive in-depth multidisciplinary integration of fundamental concepts and applications of the emerging multifunctional nanomedicines in the areas of medicine, drug delivery, and therapeutics.

In the last couple of decades, the development of new biomedical, electronic, and optical tools has provided a unique opportunity to look at materials on a nanoscale. The resultant nanotechnology boom has significantly affected the biological sciences and related areas; imaging at a subcellular level and precise delivery of drugs to tissues are two such important areas.

Nanomedicine for Drug Delivery and Therapeutics focuses mainly on the broad areas of research and applications. It presents both a comprehensive overview of the fundamental concepts as well as a practical guide for numerous applications.

This exciting new text:

- Covers the current surgical interventions to treat osteochondral defects, including the nanomaterials developed for osteochondral regeneration
- Presents the principles of regenerative medicine, the electrospinning process for the production of nanofibers
- Highlights many anticancer agents, antiviral/bacterial agents, and nucleic acids that are encapsulated in delivery nanotechnology systems
- Summarizes recent techniques for drug encapsulation, their stimuli-controlled release, passive skin permeation, and transdermal drug administration mechanisms
- Summarizes the cyclodextrin-based nano-carriers in different areas of drug delivery, particularly for oral, gene, or transdermal deliveries
- Explores current developments in gene therapy and metal-based therapy with respect to the design of effective drugs for the treatment of HIV infection
- Discusses applications of organic–inorganic hybrid bio-ceramics
- Correlates the applications of nanomedicines in diabetes management and nanotechnology
- Reviews the preparative methods of nano-phosphors, their protein-conjugates, and various physical characterizations to evaluate their possible use in membrane isolation and nano-therapeutics
- Introduces the potentiometric PVC membrane sensors using different approaches

Audience

This book is required reading for all those researchers and practitioners who are interested in a comprehensive overview of fundamental concepts and various applications in the multidisciplinary areas of medicine, drug delivery, and therapeutics. As the book covers a wide area of research that integrates biology, chemistry, physics, electronics, sensors, materials science, engineering, and nanotechnology, it also serves as an interdisciplinary guide for post-graduate researchers solving a multitude of research problems.

Ajay Kumar Mishra is currently working as the Director at the Centre for Nanomaterials Science and also as an associate professor at the Department of Applied Chemistry, University of Johannesburg, South Africa, where he is a group leader of the research area for the composites/nanocomposites, water research, and bio-inorganic chemistry.

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Nanoparticles-based Carriers for Gene Therapy and Drug Delivery

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Abstract

This chapter provides a brief overview of a variety of carriers employed for targeted delivery for use in gene and cancer therapy, summarizing the advantages and disadvantages of each approach. Particularly, our attention is aimed at magnetic particles and particles made from other materials with various surface modification (oligonucleotides, DNA, RNA, siRNA, miRNA, polyethylene glycol, poly(lactic-co-glycolic acid)). Moreover, specific attention is paid to magnetofection as one of the promising approachs for gene therapy. Further, we will briefly focus on several anticancer agents (paclitaxel, docetaxel, camptothecin, doxorubicin, cisplatin, curcumin, fluorouracil and geldanamycin), antiviral/bacterial agents (peptides, antibiotics, antivirotics) and/or nucleic acids encapsulated in a delivery nanotechnology system. The influence of their combination with the aforementioned nanoparticulate transporters, along with their properties such as cytotoxicity, short life span and solubility are discussed.

Keywords: Nanotechnologies, nanoparticles, gene therapy, anticancer drugs, drug carriers, cancer, cytostatic, targeted therapy

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16.1 Introduction

In 1974 Taniguchi used for the first time the term "Nanotechnology" which has been defined as the technology where dimensions and tolerances in the range 0.1–100 nm play a critical role. At the nano level, gravity becomes less an issue whereas the strength of materials (tolerance) becomes a greater one and also quantum size effect is an important factor. Due to the unique size-dependent spectroscopic, electronic, and thermal features, as well as chemical properties, and ability to be functionalized arising from their small sizes and high surface/volume ratios, nanomaterials found their applications not only in physics, electronics and engineering but also in life sciences including chemistry, biology and medicine. Even though nanomaterials are impacting numerous scientific fields, it can be viewed differently. In chemistry, this range of sizes has historically been associated with colloids, micelles, polymer molecules, and similar structures – typically, very large molecules, or aggregates of many molecules. More recently, structures such as fullerenes, silicon nanorods, and compound semiconductor quantum dots have emerged as particularly interesting classes of nanostructures. In physics and electrical engineering, nanoscience is most often associated with quantum behavior, and the behavior of electrons and photons in nanoscale structures. Biology and biochemistry also have a deep interest in nanostructures as components of the cell; many of the most interesting structures in biology – from DNA and viruses to subcellular organelles – can be considered as nanostructures [1–3].

16.2 Targeted Delivery

In medicine, the range of nanomaterials applications is quite broad covering both the diagnostic (imaging) as well as the treatment step.

Over the past several decades, considerable efforts have been directed towards the development of potent therapeutic agents. Yet, current anticancer therapeutics is limited in safety and efficacy. Most conventional anticancer agents show a narrow therapeutic window because they are randomly distributed in the whole body following administration. Nonspecific biodistribution may cause cytotoxicity to normal and cancer cells alike, which causes severe side effects to achieve sufficient anticancer efficacy. Moreover, nonspecific toxicity

of anticancer drugs also limits an injectable dose and thus lessens the therapeutic efficacy. To follow the fate of the drug within an organism, *in vivo* imaging systems may be used.

Nanoparticles designed for tumor targeted therapies consist of various components, in most cases from nanocarrier and an active agent (drug) [4]. Drug-carrier nanoparticles are considered as submicroscopic colloidal systems that may act as drug vehicles, either as nanospheres or nanocapsules [5]. Nanoparticle carriers are mostly composed of iron oxides, gold, biodegradable polymers, dendrimers, liposomes, viral nanoparticles and even organometallic compounds [6–8] and/or proteins. The drug encapsulation in nanocarrier provides better biocompatibility and hence its potential use in clinical oncology. Several such engineered drugs are already in clinical practice, including liposomal doxorubicin and albumin conjugate paclitaxel [9]. Concerning the nanoparticles shape, following nanostructures are frequently cited in literature: nanoshells, nanorods, nanocages, nanocubes or nanotubes.

16.2.1 Gene Delivery

The basic concept of gene therapy is to introduce into target cells a piece of genetic material that will result in either a cure for the disease or a slowdown in the progression of the disease. Gene therapy has been exploited for numerous diseases including cancer [10–12], HIV [13], cardiovascular diseases [14], metabolic disorders [15], malaria [16], spinal cord injury and/or neurological diseases [17].

The most of the research is focused on cancer treatment and numerous approaches can be taken. One of these approaches is based on selective transduction of tumor cells with a gene whose product can convert a relatively nontoxic product administered systemically to a toxic metabolite in the cancer cell. Another approach is taking advantage of selective transduction of normal cells with a gene for multiple-drug resistance. A potential advantage of this approach is that it may permit higher doses of chemotherapy to be given with less toxicity and higher efficacy. There are, however, some potential problems with this strategy because higher doses of chemotherapy may not translate into higher response rates, nonhematologic toxic effects may be dose limiting, and cancer cells may be transduced with the drug-resistance gene. Finally, tumor suppressor gene replacement and oncogene inactivation can be also utilized.

16.2.1.1 Magnetofection

Techniques for administration of the plasmid DNA are categorized into two general groups: (1) delivery mediated by a chemical carrier such as lipid-mediated delivery, peptide-mediated delivery and/or polymer-mediated delivery, and (2) naked DNA delivery by physical methods, such as electroporation, gene gun, ultrasound and magnetofection (Figure 16.1).

Nonviral gene vectors refer to synthetic reagents and additives that protect the genes from degradation and allow them to overcome cellular barriers during the delivery process. These vectors for gene delivery possess several advantages, such as enhanced biosafety and biocompatibility, high flexibility and convenience of synthesis and modification. Until now, great quantities of synthetic transfection reagents have been developed, such as cationic lipids and cationic polymers. However, the lower transfection efficiencies, compared with viral vectors, and the deficiency of biological targeting to specific tissues or cells *in vivo* still needs to be improved. In order to solve these problems, a new technology, termed as "magnetofection," has been developed. A great progress by associating magnetic nanoparticle dispersions with transfection agents such

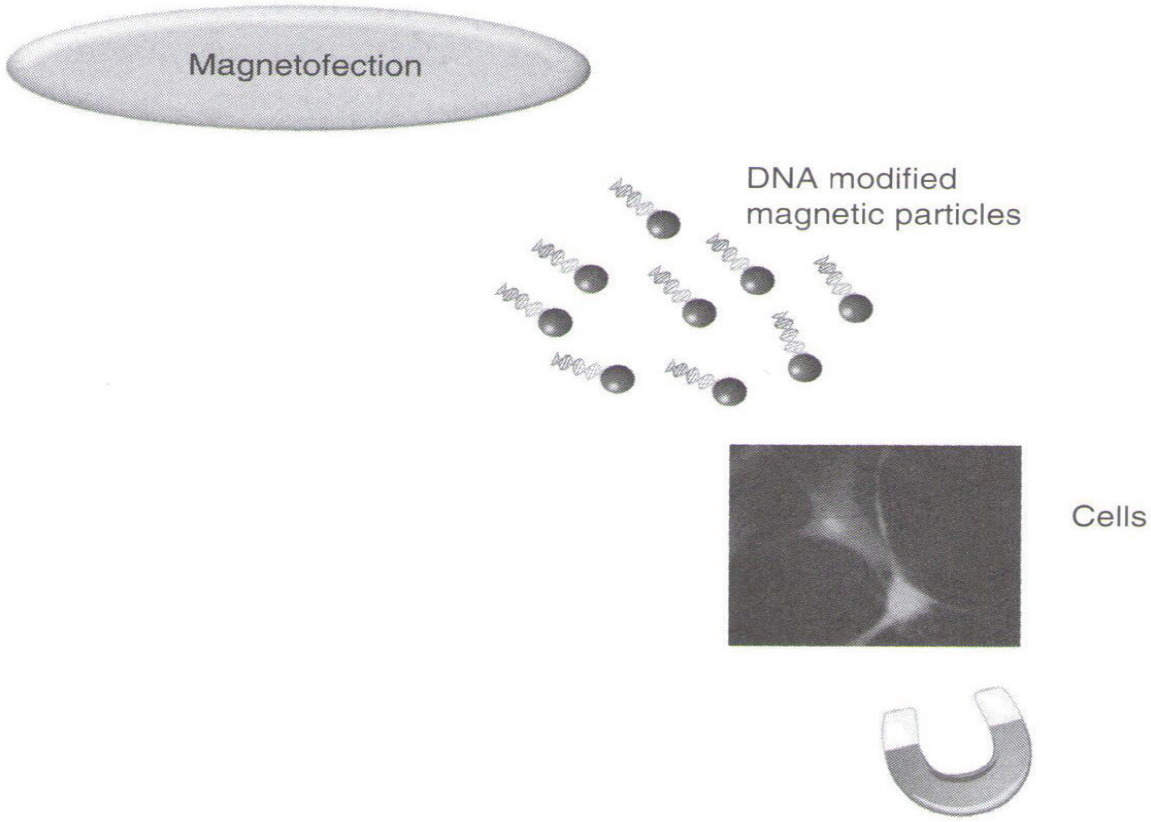

Figure 16.1 Scheme of magnetofection.

as poly(ethylenimine) (PEI) solutions has been reached and the transfection efficiency has been greatly improved. The fundamental principle of magnetofection is simple and comprises the steps of formulating a magnetic vector, adding it to the medium covering cultured cells or injecting it systemically via the blood stream or applying it locally to a target tissue, and in addition applying a magnetic field in order to direct the vector towards the target cells or retain it in the target tissue, respectively.

Magnetic nanoparticle-based transfection methods are based on the principles developed in the late 1970s by Widder and others for magnetically targeted drug delivery [18]. The use of magnetic microparticles for transfection was first demonstrated in 2000 by Cathryn Mah, Barry Byrne and others at the University of Florida, *in vitro* in C12S cells and *in vivo* in mice using an adenoassociated virus (AAV) linked to magnetic microspheres via heparin [18].

The technique is based on the coupling of genetic material to magnetic nano- (and in some cases, micro-) particles. In the case of *in vitro* magnetic nanoparticle-based transfection, the particle/DNA complex (normally in suspension) is introduced into the cell culture where the field gradient produced by rare earth magnets (or electromagnets) placed below the cell culture increases sedimentation of the complex and increases the speed of transfection. Magnetic field allows passing of MNPs through the cell membrane and reach the nucleus [19]. Chemical drug (anticancer drug, e.g., doxorubicin), biological drug (therapeutic specific proteins or peptides), nucleic acids (siRNA, antisenseRNA, DNA) and monoclonal antibodies can be anchored on MNPs to increase the selectivity of target drugs to tumor cells. *In vivo*, magnetic fields focused over the target site have the potential to not only enhance transfection but also target the therapeutic gene to a specific organ or site within the body.

The physical principles of magnetofection are virtually the same as those underlying magnetic nanoparticle-based drug targeting. This technique is based on the attractive force exerted on magnetic particles by a magnetic field source according to the equation:

$$F_{mag} = (\chi_2 - \chi_1)V\frac{1}{\mu_0}B(\nabla B) \tag{16.1}$$

where F_{mag} is the force on the magnetic particle, χ_2 is the volume magnetic susceptibility of the magnetic particle, χ_1 is the volume magnetic susceptibility of the surrounding medium, μ_0 is the

magnetic permeability of free space, V is particle volume, B is the magnetic flux density in Tesla (T), ∇B is field gradient and can be reduced to $\delta B/\delta x$, $\delta B/\delta y$, $\delta B/\delta z$.

It is clear from this equation that in order to generate a force on the magnetic particle, the magnetic field must have a gradient. In the presence of a homogeneous field, the particle will experience no force. For this reason, high-gradient, rare-earth magnets are commonly used for both magnetic nanoparticle-based drug delivery as well as for magnetofection applications [18].

16.2.2 Drug Delivery

Over the past several decades, considerable efforts have been directed towards the development of potent therapeutic agents. However, current anticancer therapeutics is limited in safety and efficacy. Most conventional anticancer agents show a narrow therapeutic window because they are randomly distributed in the whole body following administration. Nonspecific biodistribution may cause cytotoxicity to normal and cancer cells alike, which causes severe side effects to achieve sufficient anticancer efficacy. The nonspecific toxicity of anticancer drugs also limits an injectable dose and thus lessens the therapeutic efficacy.

Apatite coated bioactive and superparamagnetic particles were used and evaluated as potential materials for bone cancer treatment [20]. Zhang *et al.* synthesized tetraheptylammonium capped magnetic nano Fe_3O_4 and studied *in vitro* anticancer drug accumulation inside leukemia K562 cell lines. Observations indicated that the tetraheptylammonium-capped nano Fe_3O_4 could efficiently enhance the relevant drug permeation into cancer cells through internalization endocytosis processes and result in significantly enhanced doxorubicin uptake in relevant leukemia K562 cells [21]. Cheng *et al.* recently developed some very interesting multifunctional nanoparticles with combination of chemotherapeutic and NIR photothermal cancer therapy [22]. The complex nanosystem consists of stabilizer-free Taxol-loaded PLGA nanoparticles conjugated with amine-terminated Fe_3O_4 MNPs and PEG-grafted CdSe QDs. This nanoarchitecture was finally functionalized with poly(styrensulfonate) coated gold nanorods, which can absorb NIR light, convert it to heat and destroy PLGA nanoparticles resulting in release of encapsulated Taxol. The system was applied on HeLa cells with great efficiency. Another example of

multilayered nanoparticles combining magnetic core and two encompassing polymeric shells (PLGA and temperature sensitive poly(N-isopropyl-acrylamide) (PNIPAAm)) was published by Koppolu *et al.* [23]. Such nanocomposite can contain both hydrophilic and hydrophobic drugs, the first loaded into PNIPAAm MNPs, while second (curcumin) embedded in outer PLGA layer. Dilnawaz *et al.* conjugated in their study aqueous based protein HER2 (Human Epidermal growth factor Receptor 2) glycerol monooleate coated MNPs. The obtained results showed enhanced uptake in human breast carcinoma cell line (MCF-7), provides another potential use for highly sensitive and selective drug target for cancer HER2 positive breast cancer [24]. Multifunctional and water-soluble SPIO nanocarriers were developed by Yang and colleagues for targeted drug delivery and positron emission tomography/MRI dual-modality imaging of tumors with integrin $\alpha_v\beta_3$ cell expression. An anticancer drug was conjugated onto the PEGylated SPIO nanocarriers via pH-sensitive bonds [25].

16.2.2.1 Anticancer Drugs

The effectiveness of anticancer agents may be hindered by low solubility in water, poor permeability, and high efflux from cells. Drug delivery systems are designed to improve the safety and/or efficacy of the therapeutic agent, while simultaneously enhancing patient compliance. In these days, delivering of many drugs including the most common as paclitaxel, camptothecin, doxorubicin, cisplatin, curcumin has been tested.

Paclitaxel

Wani and colleagues found the molecule standing behind anticancer activity of bark extracts from the Pacific Yew Tree (Taxus brevifolia) and called it as "taxol" [26]. However, commercial name for this drug is paclitaxel. Nowadays, it is used for treatment of ovarian, breast, lung, head and neck, and unknown primary cancers [27]. However, poor aqueous solubility of this drug has been for more than two decades one of the main obstacle to use wider. There have been suggesting various delivery strategies including nanotechnologies in the form of polymer based controlled release systems. ReGel is an example of such system comprising of PLGA and PEG with the basic structure of PLGA–PEG–PLGA [28]. System incorporating paclitaxel into ReGel called OncoGel was designed

for local delivery of paclitaxel to solid tumors to provide targeted cytotoxicity [29]. This delivery system has been successfully utilized for treatment of several types of cancers [30–33]. Paclitaxel can be released from the ReGel for six weeks into the tumor and its surroundings. Other delivery systems including pastes, liposomes, conjugates with antibodies, peptides, and fatty acids, nanospheres and microspheres, cyclodextrin complexes, emulsions, mucoadhesive gel, prodrugs and nanoparticulate systems decreasing adverse effects of standard paclitaxel drug have been recently reviewed [34].

Camptothecin

Camptothecin (CPT) is a natural plant alkaloid extracted from Camptotheca accuminata inhibiting the activity of DNA topoisomerase I. However it is highly toxic to normal tissue cells, structurally unstable and water insoluble and therefore effective delivery to the cancer cells is challenging. Physiological conditions, such as pH equal to or above 7, causes hydrolysis of CPT leading to the opening of the lactone ring forming the inactive carboxylate. This is even more supported by binding of the human serum albumin to the carboxylate form increasing the hydrolysis yield [35]. CPT based drugs, specifically irinotecan (Camptosar) and topotecan (Hycamptin) have been approved by the Food and Drug Administration [36]. CPT has been widely used in nanoparticle mediated drug delivery studies including PEG based nanoparticles [37,38], paramagnetic Fe_3O_4 nanoparticles [39] or lipid nanoparticles [40]. Recently, synthesis of CPT loaded gold nanomaterials has been presented in the work of Xing *et al.* [35]. The authors synthesized branched nanochains consisting of spherical nanoparticles with average diameter of 10 nm. Release of CPT and the aggregation of gold nanoparticles can be controlled by tuning the solution pH.

To circumvent insolubility, unstability and toxicity of CPT, Koo *et al.* used biocompatible, biodegradable and targeted sterically stabilized micelles (SSM) as nanocarriers for CPT. They also surface-modified CPT-SSM with vasoactive intestinal peptide (VIP) for active targeting and found that CPT is efficacious against collagen-induced arthritis in mice [41].

Doxorubicin

Anthracyclines including doxorubicin (DOX) belong to antibiotics produced by Streptomyces peucetius subsp. cesius inhibiting the synthesis of nucleic acids. They are commonly used in the

treatment of a number of diverse malignant tumors – acute leukemia, non-Hodgkin's and Hodgkin's lymphoma and several solid tumors including neuroblastoma [42–46]. On the other hand, the side effect of cumulative dose dependent cardiotoxicity, myelosuppression [47] as well as large distribution volume and low life time under physiological conditions [48] represent the limitations of its clinical use. These toxic effects have been successfully reduced by employing of various nanoparticle types such as micelles, polymer based nanoparticles [49] as well as liposomes [50], and magnetic particles [51] as drug carriers.

In the study of Rai, estrogen receptor targeted liposomes encapsulating DOX was designed to enhance the delivery efficiency of doxorubicin to its destination site. The liposomal formulations showed change in the biodistribution profile. Targeted formulation accumulates more in breast and uterine tissues [52].

In the following study, Kang *et al.* examined injectable *in situ*-forming gels containing DOX as a localized drug-delivery system. These gels existed in an emulsion-sol state at room temperature and rapidly gelled *in vitro* and *in vivo* at body temperature. DOX loaded gels exhibited remarkable *in vitro* antiproliferative activities against B16F10 cancer cells. *In vivo* experiments employing B16F10 cancer cell xenograft-bearing mice showed that a single intratumoral injection of DOX loaded gel inhibited the growth of tumors as effectively as repeated injections of free DOX, and more effectively than a single dose of free DOX, or saline or gel alone [53].

Daunorubicin

Daunorubicin (DNR) is chemotherapeutic from the anthracycline family that is given as a treatment for specific types of leukemia (acute myeloid leukemia and acute lymphocytic leukemia). Like DOX, it was initially isolated from *Streptomyces peucetius*. Wang *et al.* investigated antitumor effect of daunorubicin loaded magnetic nanoparticles (DNR-MNPs) on leukemia cells *in vitro*. MNPs were spherical and their sizes were from 10 to 20 nm. The *in vitro* release data showed that the DNR-MNPs have excellent sustained release property. Proliferation of K562 cells was inhibited in a dose-dependent manner by DNR in solution (DNR-Sol) or by DNR-MNPs [54]. Li and colleagues explored the bio-application of new nanocomposites composed of poly(lactic acid) (PLA) nanofibers and Au nanoparticles as the potential nanocarrier for an efficient daunorubicin delivery into drug-sensitive K562 and drug-resistant

leukemia K562/AO2 cells. The authors observed that prepared PLA/Au nanocomposites could readily induce daunorubicin to accumulate and uptake in target leukemia cells and increase the drug's cytotoxicity [55].

Cisplatin

The biological activity of the first platinum based cytostatic drug – cisplatin (cis-diamminedichloroplatinum(II)), which is still one of the most frequently used cytotoxic agents [56–58]. The drug was discovered in 1965 by Rosenberg during his studies of an electric current effects on bacterial growth [59]. Metal organic frameworks, which is crafted from metal connecting points and organic bridging ligands can be used for encapsulation of cisplatin prodrug in [60]. Similarly, Reiter *et al.* reported cisplatin and its derivatives inclusion into nanoscale coordination polymers constructed from metal ion connectors and polydentate bridging ligands and finally stabilized against rapid dissolution in water by encapsulation in shells of amorphous silica [61]. Jain *et al.* developed hyaluronic acid-coupled chitosan nanoparticles bearing oxaliplatin encapsulated in Eudragit S100-coated pellets for effective delivery to colon tumors [62].

Curcumin

Curcumin (diferuloylmethane) is low molecular weight natural polyphenol isolated from turmeric (Curcuma longa), known for its anticarcinogenic (antiproliferative) properties and low intrinsic toxicity. Curcumin has been proved pharmacologically safe even at very high doses in many clinical studies and various animal models. However, it has extremely poor solubility in aqueous solution and moreover, it quickly degrades in neutral and alkaline conditions with a half life less than 10 min in PBS at pH 7.2, resulting in extremely low bioavailability in both vascular and oral administration [63]. To overcome these limitations, curcumin delivery by nanocarriers has been recently explored. Curcumin encapsulated in liposomes, micelles, polymeric nanoparticle, biodegradable microsphere, cyclodextrin, hydrogel nanoparticles or conjugated to water-soluble PAMAM dendrimers improved its water-solubility, stability and thus bioavailability [64]. According to Bora *et al.*, it is possible to use the composite nanoparticles consisted of three biocompatible polymers: alginate, chitosan and pluronic [65]. The other nanocarrier for curcumin was synthesized from nonionic hydrophilic methoxy-PEG shell and hydrophobic palmitate core

forming together the polymeric micelles [64]. Palmitate as lipid hydrophobic moiety was also used in combination with hydrophilic phthalimide derivative, which is potent cancer chemotherapeutic candidate. This system is able to form nanoparticles through a simple self-assembly process and then kill the cancer cells without any additional drug loading [66]. Tang *et al.* reported on the curcumin application as a comonomer to make curcumin-containing polymers (polycurcumins) by polycondensation polymerization and their *in vitro* and *in vivo* antitumor activities [63]. Another option is the usage of nanoparticulate curcumin, which is more bioavailable and has a longer half-life than native curcumin as revealed from pharmacokinetics study of Mohanty *et al.* in mice [67].

16.2.2.2 Nanoparticle Carriers

Liposomes

Liposomes belong to simplest and longest used nanocarriers [68, 69]. They are hollow structures circumscribed by phospholipid bilayer. The total size of liposome structure is about 100–150 nm. Liposomes are mainly used to solubilize drugs providing their better biodistribution compared to free drug [70, 71]. Depending on its nature, the drug is dissolved either in the lipid bilayer or in water core of liposome [72]. They are currently investigated for the delivery of vaccine, toxoids (bacterial toxin) as well as gene [73–76], anticancer [77–81], and anti-HIV drugs. Due to the ability of liposomes to withstand enzymatic cleavage, liposome-drug complex can be applied directly into circulation. However, the usage of liposomes as drug carrier is limited by rapid clearance from circulation by the reticuloendothelial system [82]. Their blood circulation time can be increased through surface modification (e.g., by attaching PEG, dextran, or poly-N-vinylpyrrolidones to the lipid bilayer). Furthermore, conjugation with targeting ligands, like monoclonal antibodies or aptamers, can enhance their tissue specificity [8].

Some important characteristics of liposomal cocktails involving the combination of two cytotoxic drugs were recently reviewed by Chiu [83]. Chen *et al.* developed As_2O_3 nanoparticles with anticancer ability encapsulated in 100 nm scale, folate-targeted liposomes to lower systematic toxicity and provide a platform for targeting this agent [84].

Bedi *et al.* studied a novel approach for intracellular delivery of siRNAs, which is potential anticancer therapeutic, into breast

cancer cells through their encapsulation into liposomes targeted to the tumor cells with preselected intact phage proteins [85]. The targeted siRNA liposomes were obtained by a fusion of two parental liposomes containing spontaneously inserted siRNA and fusion phage proteins. Recently, Abu Lila *et al.* designed PEG-coated cationic liposome to achieve dual targeting delivery of l-OHP to both tumor endothelial cells and tumor cells in a solid tumor [86]. The targeted liposomal l-OHP formulation showed an efficient antitumor activity in a murine tumor model after three sequential liposomal l-OHP injections. Cumulative cytotoxic effects of l-OHP delivered by PEG-coated cationic liposomes led to deep diffusion of a subsequent dose of liposomal l-OHP in solid tumor presumably as a result of the enlarged intra-tumoral interstitial space.

Ferritin

Ferritin is a naturally occurring molecule – protein – which is nowadays explored as a drug carrier. It was first discovered in 1937 by Laufberger, who isolated a new protein from horse spleen that contained up to 23% by dry weight of iron. Ferritin molecules are

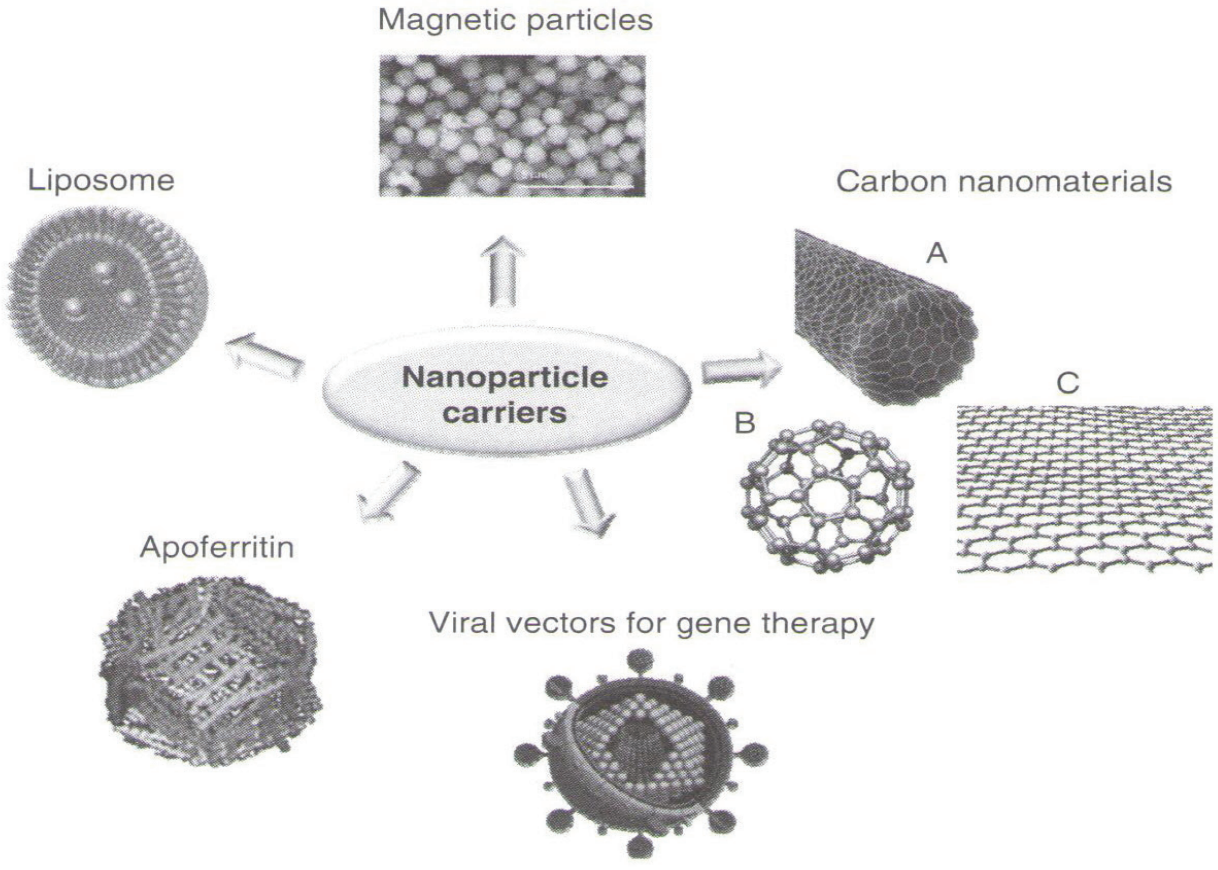

Figure 16.2 Scheme of selected nanoparticle carriers (A) carbon nanotube, (B) fullerene, (C) grapheme.

present in most living organisms and are used to store iron ions as their hydrated hydroxide-oxide Fe(III) to avoid their toxicity due to free radicals that can be generated with Fe(III), which is readily reduced to Fe(II). There are two kinds of ferritins namely maxiferritins and miniferritins. These have distinctly different inner and outer diameters and molecular weights. Maxiferritins are formed from 24 subunits 12 nm in diameter with 8 nm cavities with MW = 480 kDa and miniferritins formed from 12 subunits 8 nm in diameter with 5 nm diameter cavities of MW = 240 kDa. Mostly maxiferritins are used for study and especially horse spleen ferritin for its commercial availability. Ferritin wide occurrence as well as its ability to reversibly store and release iron ions to the living cells has attracted the interest of researchers worldwide. Ferritin consists of a protein shell with an iron oxide-hydroxide core. There can be placed around 4500 iron atoms in the structure [87]. When iron atoms are removed from ferritin protein apoferritin is obtained. Apoferritin is composed of 24 subunits of two types called H as heavy or heart of molecular weight 22 kDa and L subunits also as light or liver with molecular weight of 19 kDa. The formation of apoferritin molecule is pH dependent. When pH is lower than 3 molecule disassembles and reassembles when pH is above 5.

Nanoparticles

Due to the advances in nanomaterials numerous artificially synthesized materials are currently exploited as carriers for targeted delivery. Probably the largest group of such nanomaterials includes various nanoparticles (Figure 16.2).

Magnetic particles

The usage of magnetic nanoparticles (MNPs) made of pure iron oxide in targeted and controlled drug delivery is limited mainly due to their insufficient biocompatibility [88–90]. Therefore their modification with various materials, e.g., polymers, is unavoidable. MNPs can be used for targeting in drug and gene delivery in the case of various diseases including cancer. Magnetic field (represented by a magnet) allows passing of MNPs through the cell membrane and reach the nucleus [19]. Chemical drug (anticancer drug, e.g., doxorubicin), biological drug (therapeutic specific proteins or peptides), nucleic acids (siRNA, antisenseRNA, DNA) and monoclonal antibodies are anchored on MNPs to increase the selectivity of target drugs to tumor cells.

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Gold nanoparticles

In recent years, gold nanoparticles (AuNPs) have been studied in biological and photothermal therapeutic contexts [92,93]. This interest is motivated by the capability of AuNPs to bind a wide

range of organic molecules, their low level of toxicity, and their strong and tunable optical absorption [94]. AuNPs can be used as drug [95] and vaccine carriers into target cells or specific tissues. Generally, this has been achieved by modifying the surface of the AuNPs so that they can bind to the specific targeting drugs or other biomolecules. AuNPs can be directly conjugated with antibiotics or other drug molecules via ionic or covalent bonding [96] or by physical absorption [97]. The release of a drug from AuNPs could proceed via internal stimuli (operated within a biologically controlled manner; such as pH) or via external stimuli (operated with the support of stimuli-generating processes; such as the application of light) [98]. AuNPs size is generally about 50 nm, which is smaller than other nanomaterials like core/shell nanostructures [99,100]. For example, gum arabic glycoprotein functionalized AuNPs possess optimum sizes (core diameter of 12–18 nm and hydrodynamic diameter of 85 nm) to target individual tumor cells and penetrate through tumor vasculature and pores [101].

Gold hollow nanostructures represent a new class of metal nanomaterials suitable for anticancer drug encapsulation. These materials have ideal optical properties with strong absorption in near infrared (NIR) region (700–900 nm) which makes them attractive candidates for photothermal therapy of cancer and surface enhanced Raman spectroscopy (SERS) for *in vivo* cancer biomarker detection [102]. Photothermal therapy is a minimally invasive treatment method, where photon energy is converted to thermal energy sufficient to induce cellular hyperthermia. Selectivity is achieved by directional control or invasive positioning of the incident radiation (pulsed or continuous wave laser), and is typically accompanied by preferential administration of photoactive molecules or nano-scale particles. Photoexcitation by the laser results in non-radioactive relaxation and local heat transfer to the surrounding tumor environment [103].

Wang *et al.* prepared size-controlled supramolecular AuNPs as a photothermal agent for the targeted photothermal treatment of certain types of cancer cells [92]. They used RGD peptide ligand to target $\alpha_v\beta_3$-positive/negative cells as the corresponding biological system to test the specificity and selectivity of RGD-AuNPs. They observed selective damage of the $\alpha_v\beta_3$-positive cells and no damage of neighboring $\alpha_v\beta_3$-negative cells. The photothermal ablation of solid tumors was also investigated by Goodrich *et al.*, who tested NIR absorbing gold nanorods coated with PEG [104]. The cytotoxicity of residual cetyltrimethylammonium bromide (CTAB), which is used

in the nanorods manufacture, is also discussed regarding the presence of PEG coating [105]. They found that the diafiltration is successful method for CTAB excess removing and thus enables safety use of chosen gold nanoparticles. They also discussed possibility of AuNPs modification with antibodies against tumor cells [106].

Wang *et al.* developed a drug delivery system that tethers doxorubicin onto the surface of AuNPs with PEG spacer via an acid-labile linkage [107]. They demonstrated that multidrug resistance in cancer cells can be significantly overcome by a combination of highly efficient cellular entry and a responsive intracellular release of doxorubicin from AuNPs in acidic organelles.

Silica particles

Silica-based nanoparticles also belong to group of suitable nanocarriers for cancer treatment. These nanoparticles allow the systemic or topical administration of a photosensitive drug, so called photosensitizer (PS), into cancer cells. The researchers coated PS filled mesoporous silica nanoparticles with lipid layer to achieve cell membrane structure and biocompatible surfaces [49]. Zhu *et al.* loaded the Fe_3O_4/SiO_2 hollow mesoporous spheres with anticancer drug, doxorubicin. The authors discussed the influence of particle sizes, mesoporous shell thicknesses and concentration of Fe_3O_4/SiO_2 hollow mesoporous spheres on cell uptake and their *in vitro* cytotoxicity to HeLa cells [42].

Although the potent antitumor activity of nitric oxide (NO) supports its usage as an antineoplastic agent, effective and selective delivery and action on tumor and not normal cells remains a limiting factor. Silica nanoparticle based delivery of NO has been considered as one approach to overcome these limitations. The NO-releasing nanoparticles exhibited enhanced growth inhibition of ovarian tumor cells when compared to both control nanoparticles and a previously reported small molecule NO donor [108]. Further, it was reported the endocytosis and the time-dependent enhanced cytotoxicity of anticancer platinum drugs when the drugs were combined with (or loaded into) mesoporous silica materials [109].

Deng *et al.* investigated drug nanocarriers consisting of monodispersed and pH sensitive chitosan-silica hollow nanospheres (CS-SiO_2 HNPs) fabricated by one step method and suitable for breast cancer therapy. The resulting SiO_2 HNPs with a pH-sensitive polyelectrolyte layer are conjugated to the antibody molecule (to ErbB 2) to produce the desired nanocarriers for targeted TNF-α drug delivery to tumor cells [110].

Yuan prepared poly(acrylic acid) grafted mesoporous silica nanoparticles (PAA-MSNs) by a facile graft-onto strategy, i.e., the amidation between PAA homopolymer and amino group functionalized MSNs. The resulted PAA-MSNs were uniform spherical nanoparticles with a mean diameter of approximately 150 nm. Due to the covalent graft of hydrophilic and pH-responsive PAA, the PAA-MSNs could be well dispersed in aqueous solution, which is favorable to be utilized as anticancer drug carriers (doxorubicin hydrochloride) to construct a pH-responsive controlled drug delivery system [111].

Wang *et al.* reported on the fabrication of monodisperse hollow mesoporous silica (HMS) nanocages with uniform size possessing a hollow cubic core and mesoporous shell with penetrating pore channels based on a template-coating-etching process. The authors evaluated the therapeutic efficacy of doxorubicin loaded HMS nanocages *in vitro* and *in vivo* for liver cancer therapy. The results showed that the doxorubicin-loaded HMS nanocages have good cell uptake and can induce efficient cell death *in vitro* [107].

16.2.2.3 Carbon Materials

Both single walled (SWCNTs) and multi walled (MWCNTs) carbon nanotubes as well as a wide scale of fullerenes can be applied as carriers in specific drug delivery thanks to their unique electronic, thermal, and structural characteristics [112]. In addition, their ability to strongly absorb NIR radiation and efficiently convert absorbed energy to heat can be used for localized hyperthermia applications [113]. The researchers showed that these nanostructures can be uptaken only by cancerous cell via their functionalization with tumor-specific ligands, like radiation ion chelates, fluorescent probes folic acid and monoclonal antibodies [114,115]. Analogous to other nanostructures, the functionalization of CNTs is a key parameter to significantly reduce their toxicity and maximize the bioavailability of the anticancer drugs (proteins, nucleic acids, etc.) by improving solubility and increasing circulation time [91,116–118]. For example, Wu *et al.* combine MWCNTs functionalized with carboxylic groups with covalently attached antitumor agent 10-hydroxycamptothecin using hydrophilic diaminotriethyleneglycol as the spacer between nanotubes and drug moieties [119]. Sahoo *et al.* studied carbon nanomaterials, namely MWCNTs and graphene oxide functionalized with highly hydrophilic and biocompatible PVA for loading and delivery of an anticancer drug, camptothecin to kill human breast and skin cancer cells [120].

16.3 Conclusion

Nanotechnology is a rapidly progressing area of science which is anticipated to lead to the development of novel and multifunctional applications for diagnostics, real-time assessment of therapeutic and surgical efficacy. Utilization of nanotechnological approaches for the drug delivery is becoming an important tool in the arsenal of medicine. This multidisciplinary approach offers a great deal of flexibility in terms of ease of modification and adaptation to meet the needs of pathological conditions.

References

1. G. M. Whitesides. *Small,* Vol. 1, p. 172, 2005.
2. J. Drbohlavova, J. Chomoucka, R. Hrdy, J. Prasek, L. Janu, M. Ryvolova, V. Adam, R. Kizek, T. Halasova, and J. Hubalek. *Int. J. Electrochem. Sci.*, Vol. 7, p. 1424, 2012.
3. J. Drbohlavova, M. Vorozhtsova, R. Hrdy, R. Kizek, O. Salyk. and J. Hubalek. *Nanoscale Res. Lett.*, Vol. 7, p. 1, 2012.
4. M. Ferrari. *Nat. Rev. Cancer,* Vol. 5, p. 161, 2005.
5. L. Juillerat-Jeanneret. *Drug Discov. Today,* Vol. 13, p. 1099, 2008.
6. F. X. Gu, R. Karnik, A. Z. Wang, F. Alexis, E. Levy-Nissenbaum, S. Hong, R. S. Langer, and O. C. Farokhzad. *Nano Today,* Vol. 2, p. 14, 2007.
7. K. J. Cho, X. Wang, S. M. Nie, Z. Chen, and D. M. Shin. *Clin. Cancer Res.*, Vol. 14, p. 1310, 2008.
8. B. Mishra, B. B. Patel, and S. Tiwari. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 6, p. 9, 2010.
9. B. Haley, and E. Frenkel. *Urol. Oncol.-Semin. Orig. Investig.*, Vol. 26, p. 57, 2008.
10. T. Merdan, J. Kopecek, and T. Kissel. *Adv. Drug Deliv. Rev.*, Vol. 54, p. 715, 2002.
11. A. El-Aneed. *J. Control. Release,* Vol. 94, p. 1, 2004.
12. L. L. Nielsen, and D. C. Maneval. *Cancer Gene Ther.*, Vol. 5, p. 52, 1998.
13. M. Yu, E. Poeschla, and F. Wongstaal. *Gene Ther.*, Vol. 1, p. 13, 1994.
14. E. G. Nabel. *Circulation,* Vol. 91, p. 541, 1995.
15. M. A. Kay, and S. L. C. Woo. *Trends Genet.*, Vol. 10, p. 253, 1994.
16. F. N. Al-Deen, J. Ho, C. Selomulya, C. Ma, and R. Coppel. *Langmuir,* Vol. 27, p. 3703, 2011.
17. S. I. Jenkins, M. R. Pickard, N. Granger, and D. M. Chari. *Acs Nano,* Vol. 5, p. 6527, 2011.
18. J. Dobson. *Gene Ther.*, Vol. 13, p. 283, 2006.
19. C. Plank. *Nat. Nanotechnol.*, Vol. 4, p. 544, 2009.
20. E. M. Muzquiz-Ramos, D. A. Cortes-Hernandez, and J. Escobedo-Bocardo. *Mater. Lett.*, Vol. 64, p. 1117, 2010.

21. R. Y. Zhang, C. H. Wu, X. M. Wang, Q. Sun, B. A. Chen, X. M. Li, S. Gutmann, and G. Lv. *Mater. Sci. Eng. C-Biomimetic Supramol. Syst.*, Vol. 29, p. 1697, 2009.
22. F. Y. Cheng, C. H. Su, P. C. Wu, and C. S. Yeh. *Chem. Commun.*, Vol. 46, p. 3167, 2010.
23. B. Koppolu, M. Rahimi, S. Nattama, A. Wadajkar, and K. T. Nguyen. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 6, p. 355, 2010.
24. F. Dilnawaz, A. Singh, C. Mohanty, and S. K. Sahoo. *Biomaterials*, Vol. 31, p. 3694, 2010.
25. X. Q. Yang, H. Hong, J. J. Grailer, I. J. Rowland, A. Javadi, S. A. Hurley, Y. L. Xiao, Y. A. Yang, Y. Zhang, R. Nickles, W. B. Cai, D. A. Steeber, and S. Q. Gong. *Biomaterials*, Vol. 32, p. 4151, 2011.
26. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, and A. T. McPhail. *J. Am. Chem. Soc.*, Vol. 93, p. 2325, 1971.
27. J. Spratlin, and M. B. Sawyer. *Crit. Rev. Oncol./Hematol.*, Vol. 61, p. 222, 2007.
28. G. M. Zentner, R. Rathi, C. Shih, J. C. McRea, M. H. Seo, H. Oh, B. G. Rhee, J. Mestecky, Z. Moldoveanu, M. Morgan, and S. Weitman. *J. Control. Release*, Vol. 72, p. 203, 2001.
29. E. K. Rowinsky, E. A. Eisenhauer, V. Chaudhry, S. G. Arbuck, and R. C. Donehower. *Semin. Oncol.*, Vol. 20, p. 1, 1993.
30. N. I. Marupudi, J. E. Han, K. W. Li, V. M. Renard, B. M. Tyler, and H. Brem. *Expert Opin. Drug Saf.*, Vol. 6, p. 609, 2007.
31. C. M. Spencer, and D. Faulds. *Drugs*, Vol. 48, p. 794, 1994.
32. F. Fiorica, D. Di Bona, F. Schepis, A. Licata, L. Shahied, A. Venturi, A. M. Falchi, A. Craxi, and C. Camma. *Gut*, Vol. 53, p. 925, 2004.
33. N. L. Elstad, and K. D. Fowers. *Adv. Drug Deliv. Rev.*, Vol. 61, p. 785, 2009.
34. S. Singh, and A. K. Dash. *Crit. Rev. Ther. Drug Carr. Syst.*, Vol. 26, p. 333, 2009.
35. Z. M. Xing, Z. G. Liu, Y. G. Zu, Y. J. Fu, C. J. Zhao, X. H. Zhao, R. H. Meng, and S. N. Tan. *Appl. Surf. Sci.*, Vol. 256, p. 3917, 2010.
36. L. Brannon-Peppas, and J. O. Blanchette. *Adv. Drug Deliv. Rev.*, Vol. 56, p. 1649, 2004.
37. Y. Q. Shen, H. D. Tang, Y. H. Zhan, E. A. Van Kirk, and W. J. Murdoch. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 5, p. 192, 2009.
38. K. Ueki, H. Onishi, M. Sasatsu, and Y. Machida. *Drug Dev. Res.*, Vol. 70, p. 512, 2009.
39. A. P. Zhu, X. D. Luo, and S. Dai. *J. Mater. Res.*, Vol. 24, p. 2307, 2009.
40. Z. R. Huang, S. C. Hua, Y. L. Yang, and J. Y. Fang. *Acta Pharmacol. Sin.*, Vol. 29, p. 1094, 2008.
41. O. M. Y. Koo, I. Rubinstein, and H. Oenyueksel. *Pharm. Res.*, Vol. 28, p. 776, 2011.
42. S. J. Zhu, M. H. Hong, G. T. Tang, L. L. Qian, J. Y. Lin, Y. Y. Jiang, and Y. Y. Pei. *Biomaterials*, Vol. 31, p. 1360, 2010.
43. S. Bisht, and A. Maitra. *Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol.*, Vol. 1, p. 415, 2009.
44. K. W. Kang, M. K. Chun, O. Kim, R. K. Subedi, S. G. Ahn, J. H. Yoon, and H. K. Choi. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 6, p. 210, 2010.
45. A. Khdair, D. Chen, Y. Patil, L. N. Ma, Q. P. Dou, M. P. V. Shekhar, and J. Panyam. *J. Control. Release*, Vol. 141, p. 137, 2010.
46. D. Huska, V. Adam, P. Babula, J. Hrabeta, M. Stiborova, T. Eckschlager, L. Trnkova, and R. Kizek. *Electroanalysis*, Vol. 21, p. 487, 2009.
47. B. Manocha, and A. Margaritis. *J. Nanomater.*, Vol. Art. No. 780171, p. 9, 2010.

48. B. Asadishad, M. Vossoughi, and I. Alamzadeh. *Biotechnol. Lett.*, Vol. 32, p. 649, 2010.
49. Y. Yang, W. X. Song, A. H. Wang, P. L. Zhu, J. B. Fei, and J. B. Li. *Phys. Chem. Chem. Phys.*, Vol. 12, p. 4418, 2010.
50. C. Grange, S. Geninatti-Crich, G. Esposito, D. Alberti, L. Tei, B. Bussolati, S. Aime, and G. Camussi. *Cancer Res.*, Vol. 70, p. 2180, 2010.
51. J. Choubey, and A. K. Bajpai. *J. Mater. Sci.-Mater. Med.*, Vol. 21, p. 1573, 2010.
52. S. Rai, R. Paliwal, and S. P. Vyas. *J. Biomed. Nanotechnol.*, Vol. 7, p. 121, 2011.
53. Y. M. Kang, G. H. Kim, J. Il Kim, D. Y. Kim, B. N. Lee, S. M. Yoon, J. H. Kim, and M. S. Kim. *Biomaterials*, Vol. 32, p. 4556, 2011.
54. F. Wang, Y. C. Wang, S. Dou, M. H. Xiong, T. M. Sun, and J. Wang. *Acs Nano*, Vol. 5, p. 3679, 2011.
55. J. Y. Li, C. Chen, X. M. Wang, Z. Z. Gu, and B. A. Chen. *Nanoscale Res. Lett.*, Vol. 6, p. 8, 2011.
56. S. Krizkova, V. Adam, J. Petrlova, O. Zitka, K. Stejskal, J. Zehnalek, B. Sures, L. Trnkova, M. Beklova, and R. Kizek. *Electroanalysis*, Vol. 19, p. 331, 2007.
57. J. Petrlova, D. Potesil, J. Zehnalek, B. Sures, V. Adam, L. Trnkova, and R. Kizek. *Electrochim. Acta*, Vol. 51, p. 5169, 2006.
58. D. Huska, I. Fabrik, J. Baloun, V. Adam, M. Masarik, J. Hubalek, A. Vasku, L. Trnkova, A. Horna, L. Zeman, and R. Kizek. *Sensors*, Vol. 9, p. 1355, 2009.
59. B. Rosenberg, L. Vancamp. and T. Krigas. *Nature*, Vol. 205, p. 698, 1965.
60. R. C. Huxford, J. Della Rocca, and W. B. Lin. *Curr. Opin. Chem. Biol.*, Vol. 14, p. 262, 2010.
61. W. J. Rieter, K. M. Pott, K. M. L. Taylor, and W. B. Lin. *J. Am. Chem. Soc.*, Vol. 130, p. 11584, 2008.
62. A. Jain, S. K. Jain, N. Ganesh, J. Barve, and A. M. Beg. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 6, p. 179, 2010.
63. H. Tang, C. J. Murphy, B. Zhang, Y. Shen, E. A. Van Kirk, W. J. Murdoch, and M. Radosz. *Biomaterials*, Vol. 31, p. 7139, 2010.
64. A. Sahu, U. Bora, N. Kasoju, and P. Goswami. *Acta Biomater.*, Vol. 4, p. 1752, 2008.
65. R. K. Das, N. Kasoju, and U. Bora. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 6, p. 153, 2010.
66. N. Kasoju, D. K. Bora, R. R. Bhonde, and U. Bora. *J. Nanopart. Res.*, Vol. 12, p. 801, 2010.
67. C. Mohanty, M. Das, J. R. Kanwar, and S. K. Sahoo. *Curr. Drug Deliv.*, Vol. 8, p. 45, 2011.
68. Y. R. Hu, K. Li, L. Wang, S. S. Yin, Z. Z. Zhang, and Y. Zhang. *J. Control. Release*, Vol. 144, p. 75, 2010.
69. Y. Malam, M. Loizidou, and A. M. Seifalian. *Trends Pharmacol. Sci.*, Vol. 30, p. 592, 2009.
70. M. E. Davis, Z. Chen, and D. M. Shin. *Nat. Rev. Drug Discov.*, Vol. 7, p. 771, 2008.
71. X. Ying, H. Wen, W. L. Lu, J. Du, J. Guo, W. Tian, Y. Men, Y. Zhang, R. J. Li, T. Y. Yang, D. W. Shang, J. N. Lou, L. R. Zhang, and Q. Zhang. *J. Control. Release*, Vol. 141, p. 183, 2010.
72. V. P. Torchilin. *J. Control. Release*, Vol. 73, p. 137, 2001.
73. M. W. Epperly, S. Y. Lai, A. J. Kanai, N. Mason, B. Lopresi, T. Dixon, D. Franicola, Y. Y. Niu, W. R. Wilson, and J. S. Greenberger. *In vivo*, Vol. 24, p. 1, 2010.

74. S. Hayashi, M. Mizuno, J. Yoshida, and A. Nakao. *Cancer Gene Ther.*, Vol. 16, p. 638, 2009.
75. E. Kajiwara, K. Kawano, Y. Hattori, M. Fukushima, K. Hayashi, and Y. Maitani. *J. Control. Release*, Vol. 120, p. 104, 2007.
76. M. Nakase, M. Inui, K. Okumura, T. Kamei, S. Nakamura, and T. Tagawa. *Mol. Cancer Ther.*, Vol. 4, p. 625, 2005.
77. W. T. Al-Jamal, K. T. Al-Jamal, P. H. Bomans, P. M. Frederik, and K. Kostarelos. *Small*, Vol. 4, p. 1406, 2008.
78. D. Il Kang, H. K. Kang, H. S. Gwak, H. K. Han, and S. J. Lim. *Drug Deliv.*, Vol. 16, p. 261, 2009.
79. M. Matsui, Y. Shimizu, Y. Kodera, E. Kondo, Y. Ikehara, and H. Nakanishi. *Cancer Sci.*, Vol. 101, p. 1670, 2010.
80. N. K. Narayanan, D. Nargi, C. Randolph, and B. A. Narayanan. *Int. J. Cancer*, Vol. 125, p. 1, 2009.
81. J. Wenzel, R. Zeisig, W. Haider, S. Habedank, and I. Fichtner. *Breast Cancer Res. Treat.*, Vol. 121, p. 13, 2010.
82. N. V. Cuong, and M. F. Hsieh. *Curr. Drug Metab.*, Vol. 10, p. 842, 2009.
83. G. N. C. Chiu, M. Y. Wong, L. U. Ling, I. M. Shaikh, K. B. Tan, A. Chaudhury, and B. J. Tan. *Curr. Drug Metab.*, Vol. 10, p. 861, 2009.
84. H. Chen, R. Ahn, J. Van den Bossche, D. H. Thompson, and T. V. O'Halloran. *Mol. Cancer Ther.*, Vol. 8, p. 1955, 2009.
85. D. Bedi, T. Musacchio, O. A. Fagbohun, J. W. Gillespie, P. Deinnocentes, R. C. Bird, L. Bookbinder, V. P. Torchilin, and V. A. Petrenko. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 7, p. 315, 2011.
86. A. S. Abu Lila, Y. Doi, K. Nakamura, T. Ishida, and H. Kiwada. *J. Control. Release*, Vol. 142, p. 167, 2010.
87. G. C. Ford, P. M. Harrison, D. W. Rice, J. M. A. Smith, A. Treffry, J. L. White, and J. Yariv. *Philos. Trans. R. Soc. Lond. Ser. B-Biol. Sci.Sciences*, Vol. 304, p. 551, 1984.
88. J. Chomoucka, J. Drbohlavova, D. Huska, V. Adam, R. Kizek, and J. Hubalek. *Pharmacol. Res.*, Vol. 62, p. 144, 2010.
89. R. K. Dutta, P. K. Sharma, and A. C. Pandey. *J. Nanopart. Res.*, Vol. 12, p. 1211, 2010.
90. C. Sun, J. S. H. Lee, and M. Q. Zhang. *Adv. Drug Deliv. Rev.*, Vol. 60, p. 1252, 2008.
91. X. K. Zhang, L. J. Meng, Q. G. Lu, Z. F. Fei, and P. J. Dyson. *Biomaterials*, Vol. 30, p. 6041, 2009.
92. S. Wang, K.-J. Chen, T.-H. Wu, H. Wang, W.-Y. Lin, M. Ohashi, P.-Y. Chiou, and H.-R. Tseng. *Angew. Chem.-Int. Edit.*, Vol. 49, p. 3777, 2010.
93. N. R. Panyala, E. M. Pena-Mendez, and J. Havel. *J. Appl. Biomed.*, Vol. 7, p. 75, 2009.
94. D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel, and C. A. Mirkin. *Angew. Chem.-Int. Edit.*, Vol. 49, p. 3280, 2010.
95. P. Ghosh, G. Han, M. De, C. K. Kim, and V. M. Rotello. *Adv. Drug Deliv. Rev.*, Vol. 60, p. 1307, 2008.
96. R. Bhattacharya, C. R. Patra, A. Earl, S. F. Wang, A. Katarya, L. Lu, J. N. Kizhakkedathu, M. J. Yaszemski, P. R. Greipp. D. Mukhopadhyay, and P. Mukherjee. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 3, p. 224, 2007.

97. B. A. Rozenberg, and R. Tenne. *Prog. Polym. Sci.*, Vol. 33, p. 40, 2008.
98. D. Pissuwan, T. Niidome, and M. B. Cortie. *J. Control. Release*, Vol. in press, doi:10.1016/j.jconrel.2009.12.006 2010.
99. J. Y. Chen, C. Glaus, R. Laforest, Q. Zhang, M. X. Yang, M. Gidding, M. J. Welch, and Y. N. Xia. *Small*, Vol. 6, p. 811, 2010.
100. J. Z. Zhang. *J. Phys. Chem. Lett.*, Vol. 1, p. 686, 2010.
101. N. Chanda, P. Kan, L. D. Watkinson, R. Shukla, A. Zambre, T. L. Carmack, H. Engelbrecht, J. R. Lever, K. Katti, G. M. Fent, S. W. Casteel, C. J. Smith, W. H. Miller, S. Jurisson, E. Boote, J. D. Robertson, C. Cutler, M. Dobrovolskaia, R. Kannan, and K. V. Katti. *Nanomed.-Nanotechnol. Biol. Med.*, Vol. 6, p. 201, 2010.
102. C. W. Huang, Y. W. Hao, J. Nyagilo, D. P. Dave, L. F. Xu, and X. K. Sun. *J. Nano Res.*, Vol. 10, p. 137, 2010.
103. E. B. Dickerson, E. C. Dreaden, X. H. Huang, I. H. El-Sayed, H. H. Chu, S. Pushpanketh, J. F. McDonald, and M. A. El-Sayed. *Cancer Lett.*, Vol. 269, p. 57, 2008.
104. G. P. Goodrich, L. L. Bao, K. Gill-Sharp. K. L. Sang, J. Wang, and J. D. Payne. *J. Biomed. Opt.*, Vol. 15, p. 8, 2010.
105. E. Hutter, S. Boridy, S. Labrecque, M. Lalancette-Hebert, J. Kriz, F. M. Winnik, and D. Maysinger. *Acs Nano*, Vol. 4, p. 2595, 2010.
106. C. R. Patra, R. Bhattacharya, D. Mukhopadhyay, and P. Mukherjee. *Adv. Drug Deliv. Rev.*, Vol. 62, p. 346, 2010.
107. J. Wang, B. A. Chen, J. A. Chen, X. H. Cai, G. H. Xia, R. Liu, P. S. Chen, Y. Zhang, and X. M. Wang. *Int. J. Nanomed.*, Vol. 6, p. 203, 2011.
108. E. V. Stevens, A. W. Carpenter, J. H. Shin, J. S. Liu, C. J. Der, and M. H. Schoenfisch. *Mol. Pharm.*, Vol. 7, p. 775, 2010.
109. Z. M. Tao, B. Toms, J. Goodisman, and T. Asefa. *Acs Nano*, Vol. 4, p. 789, 2010.
110. Z. W. Deng, Z. P. Zhen, X. X. Hu, S. L. Wu, Z. S. Xu, and P. K. Chu. *Biomaterials*, Vol. 32, p. 4976, 2011.
111. L. Yuan, Q. Q. Tang, D. Yang, J. Z. Zhang, F. Y. Zhang, and J. H. Hu. *J. Phys. Chem. C*, Vol. 115, p. 9926, 2011.
112. F. Liang, and B. Chen. *Curr. Med. Chem.*, Vol. 17, p. 10, 2010.
113. S. Ghosh, S. Dutta, E. Gomes, D. Carroll, R. D'Agostino, J. Olson, M. Guthold, and W. H. Gmeiner. *Acs Nano*, Vol. 3, p. 2667, 2009.
114. R. Singh, and J. W. Lillard. *Exp. Mol. Pathol.*, Vol. 86, p. 215, 2009.
115. W. L. Qiao, B. C. Wang, Y. Z. Wang, L. C. Yang, Y. Q. Zhang, and P. Y. Shao. *J. Nanomater.*, Vol. Art. No. 796303, p. 9, 2010.
116. P. Chaudhuri, S. Soni, and S. Sengupta. *Nanotechnology*, Vol. 21, p. 11, 2010.
117. J. Liu, Z. Z. Jiang, S. M. Zhang, and W. M. Saltzman. *Biomaterials*, Vol. 30, p. 5707, 2009.
118. M. Mahmood, A. Karmakar, A. Fejleh, T. Mocan, C. Iancu, L. Mocan, D. T. Iancu, Y. Xu, E. Dervishi, Z. R. Li, A. R. Biris, R. Agarwal, N. Ali, E. I. Galanzha, A. S. Biris, and V. P. Zharov. *Nanomedicine*, Vol. 4, p. 883, 2009.
119. W. Wu, R. T. Li, X. C. Bian, Z. S. Zhu, D. Ding, X. L. Li, Z. J. Jia, X. Q. Jiang, and Y. Q. Hu. *Acs Nano*, Vol. 3, p. 2740, 2009.
120. N. G. Sahoo, H. Q. Bao, Y. Z. Pan, M. Pal, M. Kakran, H. K. F. Cheng, L. Li, and L. P. Tan. *Chem. Commun.*, Vol. 47, p. 5235, 2011.