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Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging

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Abstract

Drug delivery is an interdisciplinary and independent field of research and is gaining the attention of pharmaceutical researchers, medical doctors and industry. A safe and targeted drug delivery could improve the performance of some classic medicines already on the market, and moreover, will have implications for the development and success of new therapeutic strategies such as anticancer drug delivery, peptide and protein delivery and gene therapy. In the last decade, several drug-delivery technologies have emerged and a fascinating part of this field is the development of nanoscale drug delivery devices. Nanoparticles (NPs) have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and more recently, nucleotides. NPs and other colloidal drug-delivery systems modify the kinetics, body distribution and drug release of an associated drug. This review article focuses on the potential of nanotechnology in medicine and discusses different nanoparticulate drug-delivery systems including polymeric NPs, ceramic NPs, magnetic NPs, polymeric micelles and dendrimers as well as their applications in therapeutics, diagnostics and imaging.

From the Clinical Editor: This comprehensive review focuses on different nanoparticulate drug-delivery systems including polymeric NPs, ceramic NPs, magnetic NPs, polymeric micelles and dendrimers as well as their applications in therapeutics, diagnostics and imaging.

Key words: Nanotechnology; Drug delivery; Nanoparticles; NPs; Polymeric micelles; Dendrimers

Drug delivery is an intriguing field of research that has captured the interest of researchers because delivering a medicine to its site of therapeutic action is one of the main limitations of pharmaceutical and biotechnology industries.[1] In simple terms, drug delivery can be defined as the process of releasing a bioactive agent at a specific rate and at a specific site but in the current scenario, targeted drug delivery is a bottleneck that must be overcome to exploit thousands of new therapeutics that are limited by a safe and effective drug-delivery system.[1,2] As current advances in biotechnology and related areas are aiding the discovery and rational design of many new classes of drugs, it is crucial to improve specific drug-delivery methods to turn these new advances into clinical effectiveness. Most of the drugs are limited by their poor solubility, high toxicity, high dosage, aggregation due to poor solubility, nonspecific delivery, in vivo degradation and short circulating half-lives, but at present, the field of drug delivery is developing rapidly as researchers from various disciplines have joined in to help curtail the drugs' ever-increasing problems.[1] The field of drug delivery has also attracted the attention of the pharmaceutical industry because it offers a strategic tool to expand current drug markets; new delivery technologies could repackage classic drugs, thus offering a competitive edge after the expiration of patents and preclude competition from generics. Targeted drug-delivery systems can convey drugs more effectively and conveniently than those of the past, increase patient compliance, extend the product life cycle, provide product differentiation and reduce healthcare costs.[3] In addition, novel drug-delivery systems would offer protection and improve the pharmacokinetics of easily degradable peptides and proteins that often have short half-lives in vivo.[4,5] Therefore, the development of techniques that could selectively deliver drugs to the pathological sites is currently one of the most important areas of drug research. The emergence of nanotechnology is likely to have a significant impact on the drug-delivery sector and nanoparticles (NPs) are at the leading edge, with many potential applications in clinical medicine and research.[6] NPs can be correctly envisioned as the future of drug-delivery technology as they have the potential to become useful therapeutic and diagnostic tools in the near future. This review provides an overview of various different nanoparticulate systems that can be

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used as potential drug-delivery systems to shorten the gap between drug discovery and drug delivery. It also focuses on the potential applications of NPs for efficient drug delivery.

Nanotechnology as a solution for drug delivery

Nanotechnology is the creation and utilization of materials, devices and systems through the control of matter on the nanometer-length scale, i.e., at the level of atoms, molecules and supramolecular structures.[7,8] The applications of nanotechnology in various disciplines and specifically in healthcare are becoming increasingly common and the process of replacing traditional medicines has already begun. Thus, although efficient drug delivery is one of the most prominent problems faced by the biotechnological and pharmaceutical industries, nanotechnology can promote the innovative utilization of the myriad existing drugs produced by these industries.[9] Nanotechnology focuses on formulating therapeutic agents in biocompatible nanocarriers, such as NPs, nanocapsules, micellar systems and dendrimers (Figure 1). Moreover, one of the major advantages that nanotechnology offers is targeted drug delivery to the site of disease. This can be achieved either through passive targeting of drugs to the site of action or by active targeting of the drug (Figure 2).[10]

Passive targeting

Enhanced permeability and retention effect

Passive targeting exploits the anatomical differences between normal and diseased tissues to deliver the drugs to the required site because the physiology of diseased tissues may be altered in a variety of physiological conditions through the enhanced permeability and retention (EPR) effect.[11,12] This occurs because tumor vasculature is leaky; hence circulating NPs can accumulate more in the tumor tissues than in normal tissues. Besides exploiting the structural framework of cancerous tissues, the EPR effect is also observed at the site of inflammation. Maeda et al have demonstrated that the site of infection or inflammation where excess bradykinin is generated also exhibits the EPR-effect. The only difference between infection-induced EPR effect and that of cancer is duration of retention period; the retention in normal tissue, where inflammation occurs, is shorter than with cancer because the lymphatic drainage system is still operative; thus swelling may dissipate in a matter of a few days. In contrast, macromolecular or lipidic drug retention in cancer tissue can last weeks, a great contrast to that of infection-induced inflammation. It is interesting to note that vascular mediators and generation of such mediators triggered by cancer cells are common to inflammatory mediators; consequently, cancer is like inflammation that never ceases but grows.[13] The EPR effect has been greatly exploited for delivering various therapeutics at the site of action and many studies potentially support this mechanism of passive targeting. A number of passively targeting nanocarriers were developed in the 1980s and 1990s. One of the examples is Doxil (or Caelyx), a sterically stabilized PEGylated liposome that encapsulates doxorubicin. Doxil has shown good drug retention in the liposomal formulation with enhanced circulation time and is up to 6 times more effective in comparison with free doxorubicin.[14] It was approved for the treatment of advanced ovarian cancer, metastatic breast cancer and AIDS-related Kaposi's sarcoma. Both in animal models and in patients, such systems have been shown to result in significant improvements in reduction of tumor size, working through the EPR mechanism.[13] A poly (gamma-glutamic acid)-amphiphile complex as a novel nanovehicle for drug-delivery systems was formulated by Akao et al.[15] An in vivo assay of antitumor activity demonstrated that the complex had significant antitumor activity in sarcoma 180-bearing mice and was effectively accumulated in solid tumors based on the EPR effect, thus suggesting that the complex could be a promising formulation for targeted delivery to solid tumors and thus potentially useful in cancer chemotherapeutics. In another study, Cho et al prepared DOC (sodium deoxycholate)-heparin NPs for in vivo tumor targeting and inhibition of angiogenesis based on chemical conjugation and the EPR effect.[16] These NPs showed greater antitumor effects as well as a significant decrease in endothelial tubular formation. Recently, Chytil et al have also exploited the EPR effect for targeting HPMA copolymer-based drug carriers with covalently bound hydrophobic substituents for targeting solid tumors.[17] Treatment of mice bearing EL-4 T-cell lymphoma with the above conjugates in the therapeutic regime of drug administration via intravenous injection (IV) resulted in significant tumor regression with up to 100% of long-term survivors. These nanoconjugates also enhanced tumor accumulation, indicating an important role of the EPR effect in excellent anticancer activity of the conjugate. The tumor-targeting ability of cisplatin-loaded glycol chitosan NPs was also confirmed by Kim et al, who observed that the NPs were successfully accumulated by tumor tissues in tumor-bearing mice because of the prolonged circulation and EPR effect of NPs in tumor-bearing mice.[18]

Localized delivery

Another approach is the direct intratumor delivery of anticancer agents using NPs, which can be used in the treatment of local cancers such as prostate, head and neck cancers. Recently, Sahoo et al have demonstrated that transferrin (Tf) conjugated paclitaxel (Tx)-loaded biodegradable NPs are more effective in demonstrating the antiproliferative effect of the drug than its solution or with unconjugated Tx-loaded NPs. In human prostate cancer cell line (PC3), the IC_{50} (concentration of drug for 50% inhibition of cell growth) with Tf-conjugated NPs was fivefold lower than that with unconjugated NPs (Tx-NPs) or the drug in solution. The better efficacy of conjugated NPs was due to their greater cellular uptake and sustained intracellular retention than unconjugated NPs or the drug in solution. This characteristic of conjugated NPs maintains higher intracellular drug levels than in cells treated with drug in solution or with unconjugated NPs. Animals that received a single-dose intratumoral injection of Tf-conjugated drug-loaded NPs (Tx dose = 24 mg/kg) demonstrated complete tumor regression and greater survival rate than those that received equivalent doses of either unconjugated drug-loaded NPs or drug in Cremophor EL formulation.[19] The mechanism of greater efficacy of Tf-conjugated NPs was determined to be due to greater cellular uptake and sustained intracellular retention of the encapsulated drug than that with drug in solution or unconjugated NPs.[20] Thus, NPs are emerging as a

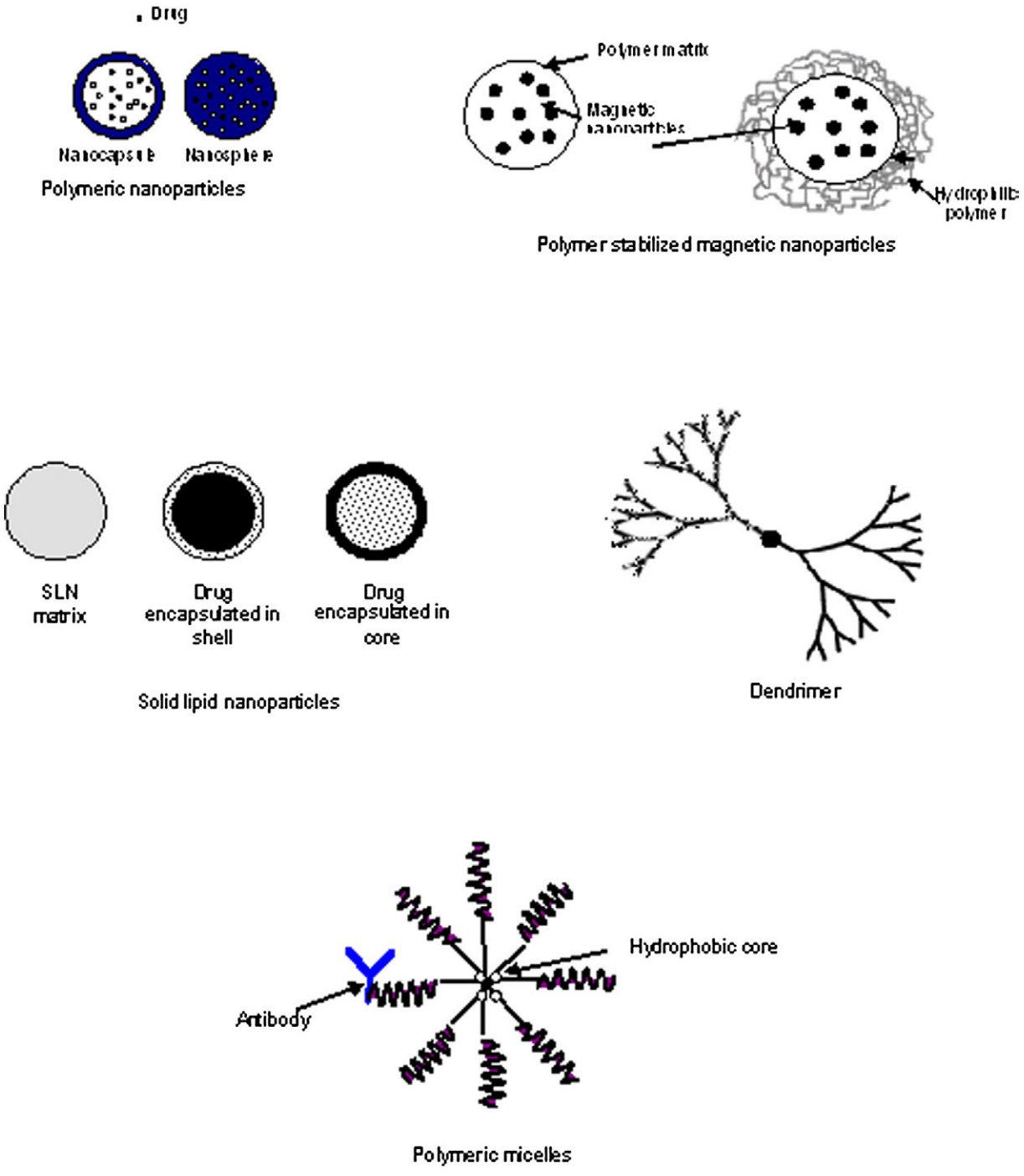

Figure 1. Schematic representation of different nanotechnology-based drug-delivery systems. Polymeric NPs are small polymeric colloidal particles with a therapeutic agent either dispersed in the polymer matrix (nanosphere) or encapsulated in polymer (nanocapsule). MNPs are nanometer-sized ferrite-or magnetite (Fe_3O_4)-based spherical particles. These particles can be coated with various hydrophilic polymers for stability and can be loaded with therapeutic agents. Solid lipid NPs are made from solid lipids (i.e., lipids that are solid at room temperature and at body temperature) and stabilized by surfactants. The drug can be encapsulated either in the shell or in the core of the NP. Dendrimers are monodispersed symmetric macromolecules built around a small molecule with an internal cavity surrounded by a large number of reactive end groups. Polymeric micelles are formed of block copolymers, which assemble in aqueous solution as outer hydrophilic layer and inner hydrophobic core. To the hydrophobic core of the micelles, water-insoluble therapeutic agents can be loaded. They can also be conjugated with different ligands and antibodies to achieve site-specific targeting.

promising tool for the intracellular delivery of practically insoluble drugs (such as taxol) and sensitive drugs (such as proteins and oligonucleotides or DNA).[21] NPs not only act as an effective carrier for such drugs protecting them from undesirable physiological conditions but also allow selective and controlled release of drugs at their target sites.

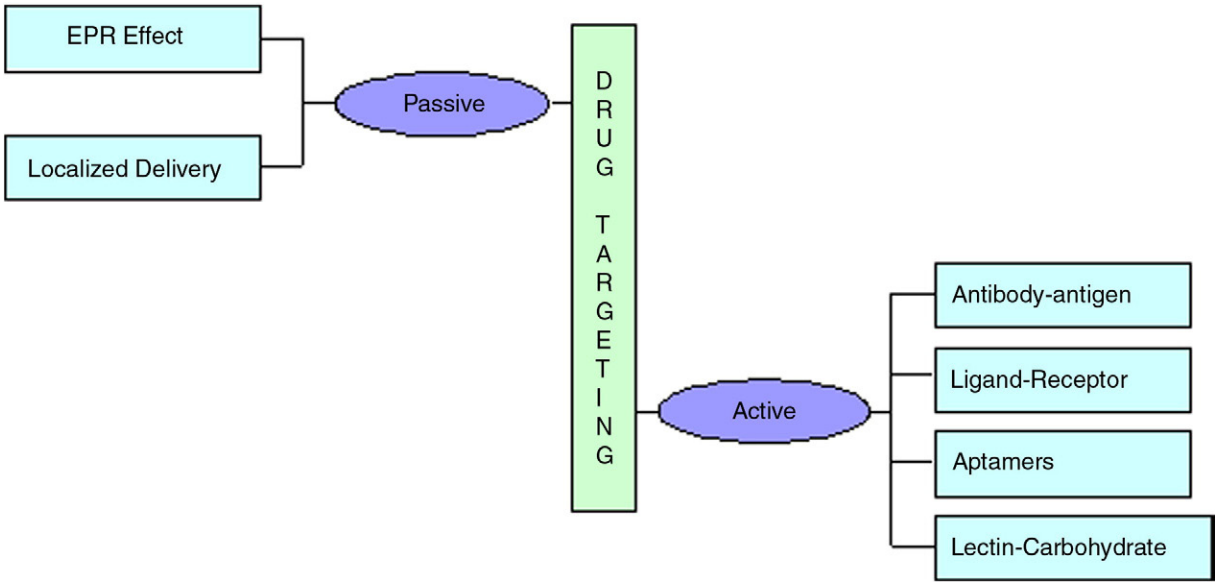

Figure 2. Schematic representation of different drug-targeting approaches.

Active targeting

Active targeting, on the other hand, requires the conjugation of receptor specific ligands that can promote site specific targeting.[22,23] The success of drug targeting depends on the selection of the targeting moiety, which should be abundant, have high affinity and specificity of binding to cell surface receptors and should be well suited to chemical modification by conjugation. The active targeting can be achieved by molecular recognition of the diseased cells by various signature molecules overexpressed at the diseased site either via the ligand-receptor, antigen-antibody interactions or by targeting through aptamers. The therapeutic agent can be actively targeted by conjugating the carrier with a cell or tissue-specific ligand, thereby allowing a preferential accumulation of the drug at the diseased site. Thus, the submicron size range of nanosystems as well as the ability to couple/conjugate different targeting ligands offers excellent opportunities to breach the physiological barriers and access different tissues followed by an efficient cellular uptake and intracellular internalization; various nanosystems can be accumulated at higher concentrations than normal drugs. PEGylated gold NPs are decorated with various amounts of human Tf by Choi et al to enhance active targeting.[24] Their results suggest that targeted NPs can provide greater intracellular delivery of therapeutic agents to the cancer cells within solid tumors than their nontargeted analogs. In another studies by Kocbek et al, cancer cells were actively targeted using PLGA NPs that were surface-modified with monoclonal antibody.[25] Their results demonstrated the superior capability of active recognition of the surface- modified NPs, because these NPs showed enhanced binding to the targeted cells than noncoated NPs. Aptamers are DNA or RNA oligonucleotide sequences that selectively bind to their target with high affinity and specificity, and these too can be exploited for active targeting of therapeutics. Tong et al synthesized aptamer-coated paclitaxel-polylactide nanoconjugates and demonstrated their enhanced targeting to cancer cells.[26]

Nanoparticles in medicine

Therapeutics

NPs have widespread use in drug delivery as already discussed with regard to the various types of NPs. Some recent applications of NP in therapeutics are discussed, possibly offering insights to the applications of NPs in therapeutics. The therapeutic applications of NPs are diverse, ranging from cancer therapeutics, antimicrobial actions, vaccine delivery, gene delivery and site-specific targeting to avoid the undesirable side effects of the current therapeutics. Many chemotherapeutic drugs such as carboplatin, paclitaxel, doxorubicin and etoposide, etc., have been successfully loaded onto NPs and these nanoparticulate systems are very potent against various cancers as demonstrated by the studies of various research groups (Figure 3). In addition, multifunctional NPs with surface-functionalized biomolecules are also being synthesized and serve as potential therapeutic agents. Functionalized NPs are also being used for targeted gene silencing because these offer exciting prospects and have garnered the attention of researchers. Many NPs are also useful as therapeutics due to their antimicrobial properties. Table 1 highlights some of the NPs that can be effectively used for therapeutics.[27-35]

Diagnostics

The drive to understand biology and medicine at the molecular level with accurate quantification demands much of current advanced analysis systems. Nanomaterials and nanotechnology combined with modern instrumentation have the potential to address this emerging challenge. This solution can be possible with the aid of a variety of nanomaterials for multiplex

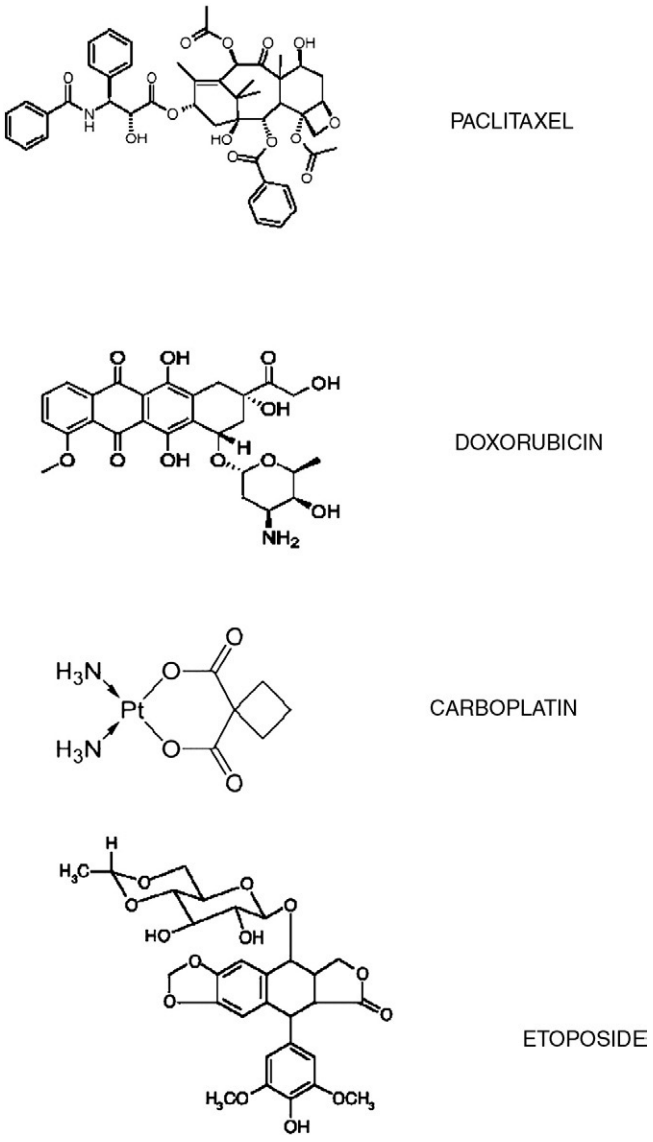

Figure 3. Chemical structure of some therapeutic drugs that can be encapsulated into NPs.

diagnostics and thus can offer sensitive, rapid and cost-effective solutions for the modern clinical laboratory. NPs are being increasingly applied to molecular diagnostics and several technologies are in development. NPs, such as gold (Au) NPs and quantum dots (QDs), are the most widely used but various other nanotechnological devices for manipulation at the nanoscale, as well as nanobiosensors, are also promising for potential clinical applications. Semiconductor QDs are NPs with intense, stable fluorescence that can enable the detection of tens to hundreds of cancer biomarkers in blood assays or on cancer tissue biopsies. They offer unique features that allow the detection of cancer markers in biological specimens at pg/mL concentrations.

Molecular diagnostics has also been greatly benefited by the advent of AuNPs which promises increased sensitivity and specificity, multiplexing capability and short turnaround times. AuNP-based colorometric assays also show great potential in point-of-care testing assays. The widespread use of AuNPs as labels in diagnostics and detection is due to a unique combination of chemical and physical properties that allow biological molecules to be detected at low concentrations. Aptamer-conjugated AuNPs has also become a powerful tool for point-of-care diagnostics.[36] Aptamer-conjugated NPs can also be used for the collection and detection of multiple cancer cells.

Thus, researchers have a high demand for simple, rapid, efficient and user-friendly alternative methods for the detection of cells in general and in particular for the detection of cancer cells. Cancer is a debilitating disease and early and accurate detection of cancer is often a bottleneck that is responsible for its delayed treatment complications. To address these limitations, various types of NPs are being developed for effective diagnostics. A brief overview is presented in Table 2.[36-42]

Imaging

The development of the effective carrier system does not only mean the execution of delivery, but also the positive confirmation of the site-specific delivery of the drug. Consequently, the ability to track and image the fate of any nanomedicine from the systemic to the subcellular level becomes essential. NPs can be successfully exploited to improve the utility of fluorescent markers for medical imaging and diagnostic purposes. Alhough various fluorescent markers are widely used in research and clinical diagnostic applications, current techniques have several disadvantages, such as the requirement of color-matched lasers, fluorescence bleaching and lack of discriminatory capacity of multiple dyes, etc. Fluorescent NPs can greatly overcome these problems and a major advance toward clinical applicability is the use of NPs to image tumors and other diseases in vivo.

Recently, fluorescent silica NPs (FSNPs), which are a new class of engineered optical probes consisting of silica NPs loaded with fluorescent dye, have also garnered immense interest in cancer imaging. The use of water-soluble, functionalized QDs that are highly stable against oxidation for biological and biomedical applications is currently one of the fastest-growing fields of nanotechnology. QDs manifest stable fluorescent properties, and also offer new prospects for live cells, in vivo imaging and diagnostics. Magnetic iron oxide NPs also have attracted extensive interest as novel contrast agents for biomedical imaging due to their capability of deep-tissue imaging, noninvasiveness and low toxicity. Dynamic magnetomotion of magnetic NPs (MNPs) detected with magnetomotive optical coherence tomography (MM-OCT) also represents a new methodology for contrast enhancement and therapeutic interventions in molecular imaging. AuNPs are also widely in use for cellular imaging. Table 3 gives an overview of the various studies on the use of functionalized NPs for imaging of diseased cells.[43-52]

Thus, different types of nanoparticulate systems can be efficiently used as in vitro and in vivo imaging agents for efficient diagnostics and therapeutics.

Advantages of NPs as drug delivery systems

NPs are submicron sized, colloidal particles, with sizes ranging from 10 – 1000 nm in diameter.[8,53] Many types of

Table 1
NPs as therapeutic agents

Type of nanomaterial	Encapsulant	Indicator	Therapeutic improvement	Reference
Polyisohexylcyanoacrylate NPs	DOX	Hepatocellular Carcinoma	Higher antitumor efficacy than native doxorubicin and can overcome multiple drug resistance phenotype.	27
PLGA NPs	Paclitaxel	Various cancers	Effective in chemotherapeutic and photothermal destruction of cancer cells	28
Gold NPs (AuNPs)	-	Various cancers	Effective as radiation sensitizers for cancer therapy	29
Chitosan NP (CNP)	siRNA	Ovarian cancer	Increased selective intratumoral delivery and significant inhibition of tumor growth compared to controls	30
Cetyl alcohol/polysorbate NPs	Paclitaxel	Brain tumor	Higher brain and tumor cell uptake, thus leading to greater cytotoxicity; also effective towards p-glycoprotein expressing tumor cells.	31
Lipid nanocapsules	Etoposide	Glioma	Greater cytotoxicity. Can overcome p-glycoprotein dependent multidrug resistance.	32
P (4-vinylpyridine) particles	-	Antimicrobial agent	These particles can be used to inhibit bacterial growth for various bacteria as biocolloids	33
Chitosan-alginate NPs	Carboplatin	Retinoblastoma	Enhanced antiproliferative activity and cytotoxicity of NPs in comparison with native carboplatin	34
Poly (3- hydroxybutyrate-co-3-hydroxyoctanoate) NPs	DOX	Various cancers	Effective in selective delivery of anticancer drug to the folate receptor-overexpressed cancer cells	35

Table 2
NPs as diagnostic agents

Type of nanomaterial	Diagnostic strategy	Advantages	Reference
AuNPs	The selectivity and specific affinity of aptamers is combined with spectroscopic advantages of AuNPs to detect diseased cells	For sensitive detection of cancer cells. Can easily differentiate between different types of target and control cells based on the aptamer	36
Magnetofluorescent particle systems	These bimodal contrast agents allows detection of cancer cells	Noninvasive diagnosis of breast cancer	37
AuNPs	Identification is based on the reaction of cell surface proteins with specific antibodies conjugated with AuNPs	Rapid identification and quantification of tumor cells	38
Semiconductor fluorescent QDs	These fluorescent biomarkers are analyzed by their resulting fluorescence and thus enables efficient cancer diagnostics	Enables fast and precise cancer diagnostics	39
Semiconductor QDs	Intense stable fluorescence enables the detection of cancer biomarkers	Useful for molecular diagnostics of cancer	40
Aptamer conjugated NPs	Aptamer-conjugated magnetic NPs can be used for selective targeting cell extraction and aptamer-conjugated fluorescent NPs can be used for sensitive cancer detection	Enables the collection and detection of multiple cancer cells	41
Fluorescent europium(III)-chelate-doped NP	Highly fluorescent europium(III)-chelate-doped NP labels, together with high affinity monoclonal antibodies (antihexon) coated on label particles and microtitration wells provides a sensitive adenovirus immunoassay	Has potential in sensitive screening of viral analytes	42

NPs can be used as drug-delivery systems and these can be formulated from diverse materials with unique architectures to serve as a possible drug-delivery vehicle to treat a particular disease. Drugs can be loaded onto NPs by various methods, such as encapsulation, surface attachment or entrapment. NPs due to their small size can efficiently penetrate across barriers through small capillaries into individual cells, thus allowing efficient drug accumulation at the target site. Therefore, the unwanted side effects and the toxicity of the therapeutic agent is reduced and the therapeutic efficacy is enhanced.[54] NPs in the pharmaceutical biotechnology sector serve to improve the therapeutic index of drugs and provide solutions to future delivery problems for new and upcoming classes of biotechnological products such as recombinant proteins and oligonucleotides. They are opening new therapeutic opportunities for therapeutic agents that cannot be used effectively as conventional drug formulations due to poor bioavailability or drug instability. The various advantages of NPs are summarized in Table 4. Because of the various advantages, diverse types of NPs can be used to deliver the active therapeutics to the site of action. However, despite widespread applications of these NPs, colloid stability in such systems has received little attention. To improve the stability of NPs, polymeric surfactants or other modifiers are often adsorbed or grafted to particles, forming a layer that generates an effective repulsive force between NPs that prevents flocculation.[55]

Table 3
NPs as imaging agents

Type of nanomaterial	Diagnostic strategy	Advantages	Reference
Poly(alkyl cyanoacrylate) NPs	The fluorescent rhodamine B-tagged poly(alkyl cyanoacrylate) amphiphilic copolymer nanoparticles enables specific human brain endothelial cell imaging	These NPs can be used for human brain endothelial cell imaging,	[43]
AuNPs	NP bioconjugates coated with dithiol bearing hetero-bifunctional PEG (polyethylene glycol), and cancer-specific monoclonal antibody F-19 can be used to label sections of healthy and cancerous pancreatic tissue.	These NPs can be used for human brain endothelial cell imaging	[44]
AuNPs	Surface functionalized AuNPs with prostate-specific membrane antigen (PSMA) RNA aptamer that binds to PSMA enables specific imaging of prostate cancer cells that expresses the PSMA protein.	Multifunctional NPs that that enables combined prostate cancer imaging by computed tomography (CT) and anticancer therapy	[45]
Streptavidin NPs	Biotinylated anti-Her2 Herceptin antibody to provide tumor targeting, whereas a biotinylated DOTA chelator labeled with ^{111}In and a biotinylated Cy5.5 fluorophore to a streptavidin NP provides specific imaging of the tumor	Streptavidin NPs were effective for multimodality imaging of tumor in mice by fluorescence and nuclear detection	[46]
Multifunctional superparamagnetic iron oxide NPs	Folate provides specific targeting, and DOX-loaded superparamagnetic iron oxide NPs serve as a therapeutic agent as well as MRI contrast agent.	Promising candidate for treating liver cancer as well as monitoring the cancer using MRI	[47]
CNPs	Tumor targeted CNPs containing dual imaging agents (near-infrared fluorescent dye, Cy5.5 (20), and gadolinium (Gd(III)) ions) was designed as dual-modality cancer imaging agents	Effective as an optical/MR (magnetic resonance) dual imaging agent for cancer treatment	[48]
QD-loaded micelles	Lipid conjugated QDs together with herceptin enhances tumor cell uptake and thus can be used for simultaneous tumor therapy and imaging	Can be used for targeting, imaging and treatment of cancer in the early stages	[49]
Carboxyl-functionalized silica-coated QDs	The stable fluorescent property of QDs enables specific imaging	Monodisperse and stable in aqueous solution, provides specific targeting and are easy for bio-conjugation. Can serve as efficient targeting probes for cell imaging	[50]
Fluorescent silica NPs	Silica NPs can be loaded with fluorescent dyes for sensitive imaging of cancer cells. In addition, for targeting cancer cells, these can be conjugated to specific biomolecules overexpressed on cancer cells	Useful for cancer targeting and imaging	[51]
Polymer-Ag@SiO2 Hybrid Fluorescent NPs	Cationic surface and suitable size allows the nanocomposites to be rapidly internalized into cells, thus effective in cellular imaging	These nanoparticles show cytocompatibility and bright fluorescence and thus is especially useful for efficient cellular imaging	[52]

Table 4
Advantages of nanoparticles

- Increase the aqueous solubility of the drug
- Protect the drug from degradation
- Produce a prolonged release of the drug
- Improve the bioavailability of the drug
- Provide a targeted delivery of the drug
- Decrease the toxic side effects of the drug
- Offer appropriate form for all routes of administration
- Allow rapid-formulation development

Polymeric NPs

Most polymeric NPs are biodegradable and biocompatible,[19] and over the past few decades, researchers have had considerable interest in developing biodegradable NPs as a drug- delivery system.[8,21] Moreover, they also exhibit a good potential for surface modification and functionalization with different ligands, provide excellent pharmacokinetic control and are suitable to encapsulate and deliver a plethora of therapeutic agents. The chemical structure of the various polymers that can be used for the formulation of the polymeric NPs is depicted in Figure 4.[25] Depending on the process used for their preparation, these can be NPs, nanospheres or nanocapsules. Nanospheres have a matrix-like structure, where active compounds can be firmly adsorbed at

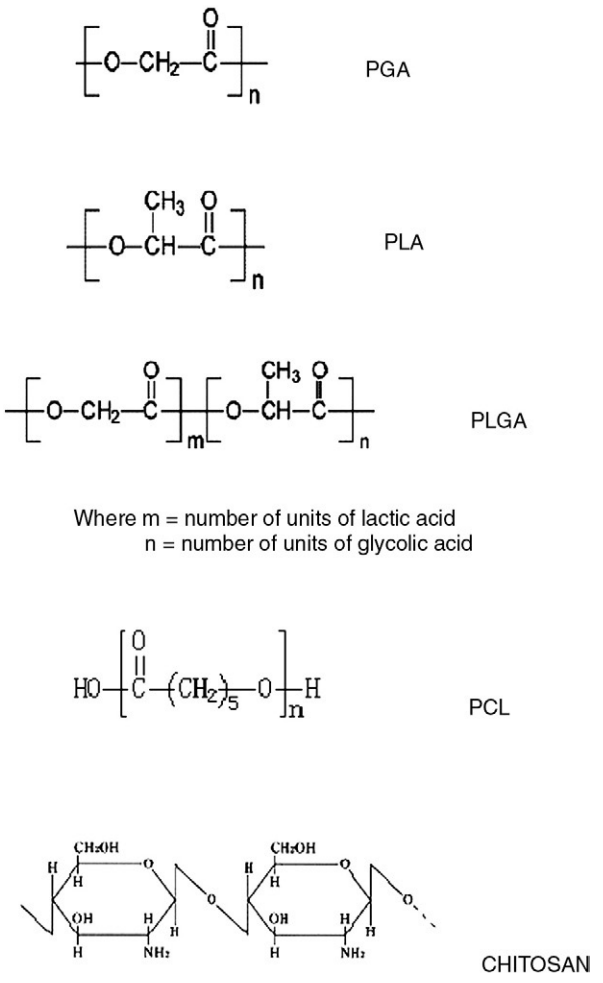

Figure 4. Some polymers used for the formulation of NPs.

their surface, entrapped or dissolved in the matrix. Nanocapsules have a polymeric shell and an inner core. In that case, an active substance is usually dissolved in the core but can also be adsorbed at their surface.[8,21] The main advantage of using NPs for drug-delivery applications is their small size when taken up by cells, which could allow efficient drug accumulation at the target sites.[56] Biodegradable materials used for the formulation of NPs allow sustained drug release within the target site over a period of days or even weeks. Biodegradable NPs formulated from poly D, L-lactide *co*-glycolide (PLGA) and polylactide (PLA) have been investigated for sustained drug delivery.[21,57] The main interest of researchers is to study their intracellular trafficking and to determine the parameters that are critical to their efficient cellular uptake and retention. Recently, studies have demonstrated rapid escape of NPs from the endo-lysosomal compartment to the cytoplasmic compartment.[57] Greater and sustained antiproliferative activity of paclitaxel-loaded PLGA NPs in HeLa cells was observed by the research group of Yang et al. Enhanced apoptosis of HeLa cells was observed, which may be due to the sustained release of paclitaxel from the PLGA NPs, which in turn showed that PLGA NP-encapsulated paclitaxel is promising as a controlled drug-delivery system in future clinic application.[58] Recently, NPs formulated from PLGA were investigated as a drug-delivery system to enhance tissue uptake, permeation and targeting of zinc (II) phthalocyanine (ZnPc) for photodynamic therapy. Tumor-bearing mice injected with ZnPc NPs exhibited significantly smaller mean tumor volume, increased tumor growth delay and longer survival in comparison with the control group and the group injected with free ZnPc during the time course of the experiment. Histopathological examination of tumor from animals treated with PLGA ZnPc showed regression of tumor cells, in contrast to those obtained from animals treated with free ZnPc. The results indicate that ZnPc encapsulated in PLGA NPs is a successful delivery system to improve photodynamic activity in the target tissue.[59]

Multidrug resistance (MDR) is one of the major causes of treatment failure in cancer therapy, which may be attributed to the decreased accumulation of drug in the tumor site in addition to the possibility of membrane glycoprotein (P-gp)-dependent accelerated drug efflux.[60,61] To overcome the problem of efflux action of P-gp and to sustain drug effect, various drug-delivery systems have been developed. PLGA NPs formulations capable of delivering a cytotoxic drug, vincristine, a chemosensitizer, verapamil or their combination were prepared by the research group of Song et al. The results showed that PLGA NPs simultaneously loaded with anticancer drug and chemosensitizer might be the one of the potential formulations in the treatment of drug-resistant cancers in vivo as the simultaneous administration of vincristine and verapamil could achieve the highest reversal efficacy on MCF-7/ADR cells resistant to vincristine.[62] In other studies, Wang et al developed an efficient and targeted delivery of antisense oligodeoxynucleotides (asODNs), using folic acid (FA)-conjugated hydroxypropyl-chitosan (HPCS) NPs to reduce production of P-gp to overcome tumor drug resistance. The FA-HPCS-asODNs NPs demonstrated significant inhibition of the MDR 1 gene levels and P-gp levels in vitro and in vivo, respectively, in comparison with asODNs and HPCS-asODNs alone. Thus, these results suggest that the use of targeted, antisense agent NPs would be a potential approach to overcome tumor drug resistance.[63]

Another characteristic function of NPs is their ability to deliver drugs to the target sites across biological barriers such as the blood-brain barrier (BBB).[64,65] The brain delivery of a wide variety of drugs, such as antineoplastic and anti-HIV drugs, is markedly hindered because they have great difficulty in crossing the BBB.[66] Thus, by using the nanotechnological approaches, researchers have tried to improve the pharmacokinetics of drugs for the treatment of central nervous system (CNS) diseases. The application of NPs to brain delivery is a promising way to overcome this barrier. Kreuter and colleagues demonstrated that poly-(butylcyanoacrylate) NPs coated with polysorbate-80 are effective in carrying different drugs to the brain.[67] Although not fully elucidated, the most likely transport mechanism for these particles is via endocytosis across the endothelial cell lining of the BBB. Moreover, by packaging therapeutic molecules inside a liposome and decorating the surface of the liposome using molecular “Trojan horse” technology, researchers have obtained promising results. For example, OX-26-transferrin-targeted poly (ethylene glycol) (PEG)-ylated immunoliposomes carrying expression plasmids of the gene-encoding tyrosine hydroxylase

have been employed successfully in a rat model of Parkinson's disease.[68] Trimethylated chitosan (TMC) surface-modified (PLGA) NPs (TMC/PLGA-NP) were synthesized by Wang et al as a drug carrier for brain delivery. In their studies, they confirmed the brain-targeted effects of TMC/PLGA-NP and demonstrated that TMC surface-modified NPs are able to cross the BBB and appear to be a promising brain drug-delivery carrier with low toxicity.[60,69]

Recently many surface-modified NPs are being used to treat various diseases. Surface modification of PLGA NPs with polyethyleneimine (PEI) utilizing a cetyl derivative was used to improve surface functionalization and aid siRNA delivery. Specific reduction in the anti-apoptotic oncogene BCL-w in U2OS cells was achieved with particles containing cetylated-PEI with no apparent cellular toxicity. In addition, particles containing cetylated-PEI achieved 64% silencing of TNF alpha in J774.1 cells.[70] Sahu et.al have prepared hydrophobically modified carboxymethyl chitosan NPs for targeted delivery of paclitaxel. Target-oriented NPs based on biodegradable O-carboxymethyl chitosan modified with stearic acid and surface modified by covalent attachment of FA to achieve tumor cell targeting properties were prepared and characterized and these showed excellent cytotoxic property in comparison with the native drug.[71]

Solid-lipid NPs

Solid lipid NPs (SLN) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes and polymeric NPs as a colloidal carrier system for controlled drug delivery.[6] These particles are made from solid lipids (i.e., lipids that are solid at room temperature and also at body temperature) and stabilized by surfactant(s). SLN can be formulated by using highly purified triglycerides, complex glyceride mixtures or even waxes. In comparison with other particulate carriers, SLN has many advantages for drug delivery, such as good tolerability, biodegradability,[72] a high bioavailability by ocular administration[73] and a targeting effect on the brain.[74] In recent years, the study of SLN has markedly increased, especially with the method of high pressure homogenization. SLN have been developed and investigated for parenteral, pulmonary and dermal application routes.[75-77] Because of their small size, SLN may be injected intravenously and used to target drugs to particular organs. The particles together with all intravenously injected and colloidal particulates are cleared from the circulation by the liver and spleen. In tumor tissues, drugs can be targeted by PEG-coated polymeric NPs (known as stealth property) that helps in escaping from reticuloendothelial system (RES).[78] This may be achieved using block polyoxyethylene polypropylene copolymers like Pluronic F188 in which the hydrophobic portion of the molecule forms the NP matrix while the water soluble polyoxyethylene block forms a hydrophilic coating on the particle. Stealth SLN increases the tumor accumulation, antibacterial activity of antiparasitic and antifungal drugs, and allows brain delivery of anticancer drugs not capable of crossing the BBB.[79] Recently SLNs have also been used for the targeted delivery of therapeutics to the alveolar macrophages by Yu et al. In their study, a mannan-based PE-grafted ligand was synthesized and used for the surface modification of DNA-loaded cationic SLN to prepare Man-SLN-DNA. Their results showed that in comparison with nonmodified SLN-DNA and Lipofectamine 2000-DNA, Man-SLN-DNA produced the highest gene expressions, especially in vivo. Thus, these modified SLNs may have great potential for targeted gene delivery.[80]

Ceramic NPs

The use of inorganic (ceramic) particles for drug delivery, especially biomacromolecular therapeutics, is emerging as a new area.[81] Ceramic NPs because of their ultra-low size (less than 50 nm) and porous nature are becoming important drug-delivery vehicles. It is well documented that smaller-sized drug-carrier systems are more effective in evading the uptake by the RES.[82] In addition, ceramic NPs do not show swelling or changes in porosity with pH. Therefore, these particles can effectively protect different biomacromolecules, such as enzymes, against denaturation induced by changes in the external pH and temperature. Silica and other materials such as alumina, titania, etc., which are highly compatible with biological systems because of their inert nature, are widely being used for the formulation of NPs. Further their surfaces can be easily functionalized for conjugation to target-specific ligands such as monoclonal antibodies.[83-84] It was recently reported by Li et al that Poly-L-lysine-modified silica NPs (PMS-NPs) are a novel nonviral vector for gene delivery.[85] Roy et al reported a novel NP-based drug carrier for photodynamic therapy.[86] The group synthesized ultra-fine organically modified silica-based NPs (diameter ~30 nm), entrapping water-insoluble photosensitizing anticancer drug and 2-devinyl-2- (1-hexyloxyethyl) pyropheophorbide in the nonpolar core of micelles formed by hydrolysis of triethoxyvinylsilane. The resulting drug-doped spherical monodispersed NPs demonstrated an uptake into the cytosol of the tumor cells that, when irradiated, generated singlet oxygen and damaged tumor cells. Silica NPs have been also tested as a nonviral vector for gene delivery.[87] Recently, it has been evaluated that NPs made up of some metal oxides possess therapeutic properties themselves. Mishra et al has reported that a novel Ag+-loaded zirconium phosphate NP plays vital bacteriostatic roles in diabetic wound healing.[88] Moreover, NPs made of cerium oxide (CeO_2, ceria), aluminum oxide (Al_2O_3, alumina) and Yttrium oxide (Y_2O_3, yttria) were also considered for their potential scavenger behavior. Three alternative explanations were demonstrated for the scavenging property of these NPs: they may act as direct antioxidants, block ROS production and directly cause a low level of ROS production.[89] Schubert et al have evaluated therapeutic properties of CeO_2 and Y_2O_3 particles by using HT22 cells derived from the rodent nervous system. They have shown that NPs composed of CeO_2 and Y_2O_3 have antioxidant properties that promote cell survival under conditions of oxidative stress leading to protection of cells from death. It tracks that there is a potential for engineering this group of NPs for therapeutic purposes.[90] Bharali et al reported application of organically modified silica (ORMOSIL) NPs as a nonviral vector for efficient in vivo gene delivery. They prepared and characterized highly monodispersed, stable aqueous suspension

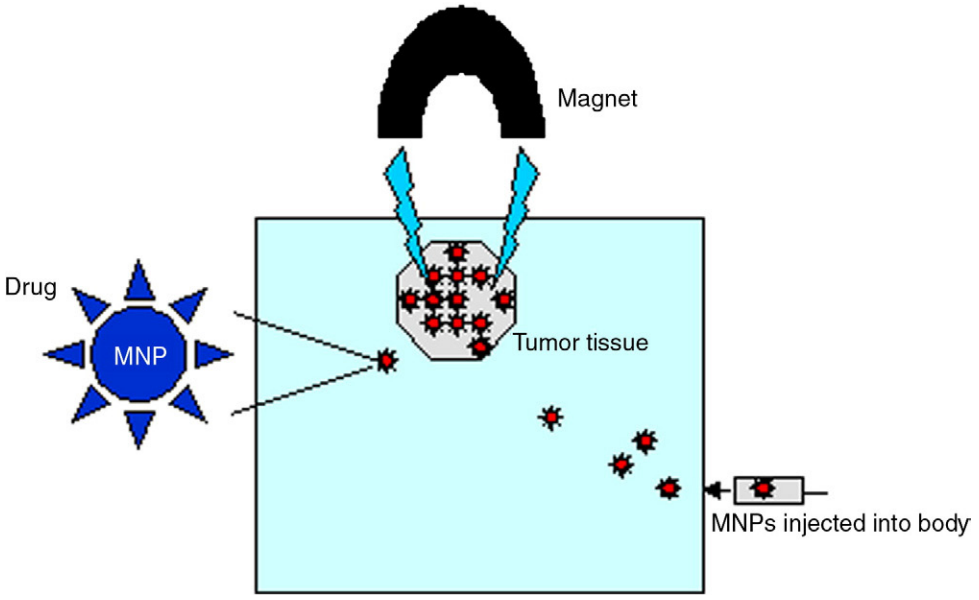

Figure 5. MNPs specifically targeted to the tumor tissue with the help of an external magnetic field.

of NPs, surface-functionalized with amino groups for binding of DNA. The ORMOSIL-mediated transfections were used to manipulate the biology of the neural stem progenitor cells in vivo. Transfection of a plasmid expressing the nucleus-targeting fibroblast growth factor receptor type 1 resulted in significant inhibition of the in vivo incorporation of bromodeoxyuridine into the DNA of the cells in the subventricular zone and the adjacent rostral migratory stream. This in vivo approach shows that the nuclear receptor can control the proliferation of the stem progenitor cells in this region of the brain. The results of this nanomedicine approach using ORMOSIL NPs as a nonviral gene-delivery platform have a promising future direction for effective therapeutic manipulation of the neural stem progenitor cells as well as in vivo targeted brain therapy.[91] Ceramic composites are also increasingly being considered as third-generation orthopedic biomaterials due to their ability to closely match the chemical, biological and mechanical properties of natural bone. The studies by Liu et al demonstrated that the combination of PLGA with well-dispersed nanoceramics (titania and hydroxyapatite) enhanced mechanical properties necessary for load-bearing orthopedic and dental applications. It also enhanced the tensile modulus, tensile strength at yield, ultimate tensile strength and compressive modulus.[92]

Magnetic NPs

Magnetic NPs (MNPs) are increasingly being realized as one of the most important materials in the industrial sector, and they are being widely used for biotechnological and biomedical applications. Drug delivery may be immensely benefited by the use of MNPs because these particles have the ability to target a specific site, such as a tumor, thereby reducing the systemic distribution of cytotoxic compounds in vivo and enhancing uptake at the target site, resulting in effective treatment at lower doses.[93] Magnetic fluids are stable colloidal suspensions of MNPs dispersed in organic or inorganic liquid carriers. In the preparation of colloidal MNPs, the stability of the colloid is of utmost importance. Magnetic iron oxide particles without any surface coatings have hydrophobic surfaces with a large surface area to volume ratio, this leads to particles' agglomeration and formation of large clusters, resulting in increased particle size. This inherent aggregation behavior of MNPs is a crucial limiting factor that reduces the intrinsic superparamagnetic properties and triggers the opsonization process.[94] Therefore, to minimize the aggregation, it is necessary to engineer the surface of the MNPs. Synthetic and natural polymers (dextran, polyethyleneglycol (PEG), and poly(vinylpyrrolidone) (PVP), streptavidin, poly-L-lysine (PLL), polyethylene imide (PEI), etc.,) have been employed to modify the surface of the MNPs.[95] Sahoo et al have reported the stabilization of NPs in organic solvents by surface-derivatized magnetite by oleic acid, lauric acid, dodecylphosphonic acid, hexadecylphosphonic acid, dihexadecylphosphonic acid, etc.[96] They found that alkyl phosphonates and phosphates could be used to obtain thermodynamically stable dispersions of MNPs. Surface coverage by amphiphilic polymeric surfactants such as poloxamers, poloxamines and PEG derivatives over the MNPs significantly increases the blood circulation time by minimizing or eliminating the protein adsorption to the NPs. In an attempt, Zhang et al have prepared the superparamagnetic MNPs by surface modified with PEG to resist the protein adsorption and thus avoid the process of opsonization and also to facilitate the intracellular uptake by specific cancer cells for cancer therapy and diagnosis.[97]

Magnetic-based delivery strategies are based on binding drugs with magnetic fluids that concentrate the drug in the site of interest. In magnetic drug targeting, magnetic carrier particles with surface-bound drugs are injected into the vascular systems that are then captured at the tumor via a locally applied magnetic field (Figure 5). The surface-bound drugs can be released from the drug carriers by changing the physiological conditions, and

are then taken up by the affected cells. Mainly two types of iron oxide (magnetite, Fe_3O_4 and maghemite, Fe_2O_3) have been used for various biomedical applications and of these two types, magnetite is a very promising candidate because of its biocompatibility. Another advantage is that both Fe_3O_4 and γ-Fe_2O_3 when produced in nanoparticulate form exhibit superparamagnetic behavior at room temperature; i.e., they magnetize strongly under an external magnetic field but retain no permanent magnetism once the field is removed. This quality leads to easy dispersal, as the particles are unlikely to clump together. In recent years, various functionalized MNPs for use in several biomedical applications, such as drug delivery, magnetic separation and magnetic resonance imaging (MRI) contrast agents for diagnostics have emerged. The aim of the surface modification is not only to stabilize the NP suspension in vitro and govern their in vivo fates but also to minimize remnant magnetization. Precoating of the MNPs also makes them biostable, biodegradable and nontoxic. Various magnetizable nanophases (e.g., magnetite, maghemite, cobalt, etc.) are also being incorporated into different biocompatible and nontoxic polymers, such as PLGA, PLA, dextran and chitosan, etc., and these specific carriers can also be utilized for targeted drug delivery, as these nanophases provide the carriers with the unique characteristics of being noninvasively and selectively guided to and concentrated within the desired body location using internal or external magnetic fields. The most promising application of these colloidal magnetic NPs is for site-specific drug delivery. These NPs can carry therapeutic agents on their surface or in their bulk when formulated using polymers, which could be driven to the target organ under an external magnetic field and then released there. For these applications, the size, charge and surface chemistry of magnetic particles are particularly important because these properties strongly affect both their blood circulation time as well as bioavailability of the particles within the body.[98] In addition, magnetic properties and internalization of particles in the target tissue depend strongly on the size of the magnetic particles.[99] For example, following systemic administration, larger particles with diameters greater than 200 nm are usually sequestered by the spleen as a result of mechanical filtration and are eventually removed by the cells of the phagocyte system, resulting in decreased blood circulation time. On the other hand, smaller particles with diameters less than 10 nm are rapidly removed through extravazations and renal clearance. Particles ranging from 10 to 100 nm are optimal for intravenous injection and demonstrate the most prolonged blood circulation times. MNPs in the above size range are small enough both to evade the RES of the body as well as penetrate small capillaries within the body tissues and, therefore, may offer the most effective distribution in certain tissues.

Several studies have demonstrated application of MNPs for drug delivery. Novel hybrid MNPs with average diameter of less than 160 nm and comprising hyaluronic acid (HA) and iron oxide was synthesized and characterized by Kumar et al. These particles were further tested for their ability to deliver peptides to the cells using HEK293 and A549 cells. Their results showed that the NPs delivered peptides at about 100% level and that these particles are expected to be useful in developing effective tissue and cell targeting systems.[100] Liu et al developed high magnetization, biodegradable/biocompatible polymer-coated magnetic nanospheres for biomedical applications. These magnetic nanospheres not only showed good encapsulation efficiency, they also showed high magnetite content (40%–60%) and had high magnetization (26–40 emu/g). Thus, these biodegradable nanospheres are suitable as a potential platform for the design of magnetically guided drug delivery and other in vivo biomagnetic applications.[101] Jain et al developed a novel water-dispersible oleic acid (OA)- Pluronic-coated iron oxide NPs formulation for the sustained delivery of high doses of anticancer agents. Sustained release of the incorporated drug, doxorubicin, was observed over a period of 2 weeks and the NPs also demonstrated a sustained intracellular drug retention relative to drug in solution and a dose-dependent antiproliferative effect on breast and prostate cancer cell lines.[102]

Recently, polyethyleneimine (PEI)-modified magnetic NPs (GPEI) have been developed as a potential vascular drug/gene carrier to brain tumors wherein intracarotid administration in conjunction with magnetic targeting resulted in 30-fold (P = .002) increase in tumor entrapment of GPEI in comparison with that seen with intravenous administration.[103] In another study, Si et al has improved the antitumor effect of genistein with a biocompatible superparamagnetic drug-delivery system, which is based on covalently attaching genistein onto Fe_3O_4 NPs coated by cross-linked carboxymethylated chitosan (CMCH). Their studies demonstrated that Fe_3O_4-CMCH-genistein nano-conjugate exhibited a significantly more enhanced inhibition effect on the SGC-7901 cancer cells than did the free genistein; thus, this drug-delivery system could be promising for future multifunctional chemotherapeutic application that combines drug release and magnetic hyperthermia therapy.[104] In another interesting study, Sarin et al prepared functionalized dendrimers that could effectively cross the BBB and accumulate in malignant glioma cells. Magnetic resonance and fluorescence imaging probes were conjugated to the dendrimer to track the transvascular transport and localization of the NPs within glioma cells. It was found that intravenously administered functionalized dendrimers smaller than approximately 11.7 to 11.9 nm in diameter were able to traverse pores of the blood-brain tumor barrier, and larger ones could not. Thus, an effective drug-delivery system designed for malignant glioma cells should be smaller than 11.7 to 11.9 nm in diameter and possess long blood half-lives.[105]

Thus, MNPs can be successfully used for a wide range of drug-delivery applications and MNPs may pave the way for new and unconventional approaches toward the improved management and general health by an advancement of early diagnosis of many killer diseases.

Metal based NPs

Metal NPs can be synthesized in extremely small sizes of around 50 nm and thus the large surface area provides the ability to carry a relatively higher dose of drugs. AuNPs are most commonly used as they offer manifest advantages. It is easy to synthesize a range of sizes of Au NPs by changing simple parameters by simple, cheap and reliable methods. Moreover, due to the presence of negative charge on gold NPs, these can be easily functionalized by various biomolecules.[106] An additional

advantage is that it is biocompatible and nontoxic.[107] AuNPs are also useful due to their unique physicochemical properties, such as ultra small size, large surface- area-to-mass ratio, high surface reactivity and the presence of surface plasmon resonance (SPR) bands. SPR is responsible for their large absorption and scattering cross-sections, which are 4 to 5 orders of magnitude larger than that of conventional dyes.

AuNPs also have a variety of drug-delivery applications. Joshi et al exploited AuNPs as carriers for efficient transmucosal insulin delivery. Their results showed that there was a significant reduction of blood glucose levels (postprandial hyperglycemia) when insulin was delivered using AuNPs as carriers by the transmucosal route in diabetic rats, thus establishing these as potent drug-delivery vehicles.[108] Multifunctionalized AuNPs with peptides targeted to gastrin-releasing peptide receptors of a tumor cell line was successfully synthesized by Hosta-Rigua et al whose research showed an enhancement of the activity and selectivity of the peptide multifunctionalized conjugates.[109] AuNPs for the improved anticancer drug delivery of oxaliplatin were synthesized by Brown et al, That synthesis showed enhanced cytotoxicity in all the cell lines tested and the AuNPs showed an unusual ability to penetrate the nucleus in the lung cancer cells.[110] Functionalized AuNPs could also play an important role in efficient drug delivery and biomarking of drug-resistant tumor cells as was evident from the studies of Li et al. Functionalized AuNPs (3-mercaptopropionic acid capped gold NPs) were effective in drug delivery to drug-resistant leukemia K562/ADM cells. This could be explored as a novel strategy to inhibit multidrug resistance in targeted tumor cells.[111]

Currently many other metal-based NPs, as well as various hybrid surface-functionalized NPs, are being extensively used for drug-delivery applications. New hybrid systems consisting of anticancer drugs, such as methotrexate (MTX) or 5-fluorouracil (5-FU) and carrier-like layered double hydroxide (LDH), which are inorganic vectors with biocompatible metal ions, were developed by Choi et al. These LDH NPs demonstrated sustained drug release, prolonged drug half-life and increased drug accumulation in targeted tumor tissue. Thus, these hybrid systems can be promising anticancer chemotherapy agents for tumor targeting with biocompatibility.[112] Silver NPs have wound-healing properties as was demonstrated by Tian et al who found that rapid healing and improved cosmetic appearance occurred in a dose-dependent manner by the topical delivery of silver NPs. These NPs also exert positive effects through their antimicrobial properties, reduction in wound inflammation and modulation of fibrogenic cytokines.[113] Amphiphilic TiO_2 nanotube arrays can serve as an actively controllable drug-delivery system by utilizing the photocatalytic ability of TiO_2, a precisely controlled removal of the cap and a highly controlled release of the hydrophilic payload (drug) can be achieved.[114]

Polymeric micelles

Polymeric micelles represent a class of micelles that are formed of block copolymers consisting of hydrophilic and hydrophobic monomer units.[115] These particulates are composed of a core of hydrophobic blocks stabilized by a corona of hydrophilic polymeric chains. Although a variety of hydrophilic polymers can be used but in majority of cases, PEG blocks with a molecular weight ranging from 1 to 15 kDa are used as corona-forming blocks, and the length of a hydrophobic core-forming block is close or somewhat lower than that of a hydrophilic block.[116] In broad terms, a micellar system as drug carrier provides a set of unsurpassable advantages over other methods.[117] Enhancing drugs' solubility using micelle-forming surfactants results in increased water solubility of a poorly soluble drug. They also improve drugs' bioavailability by enhancing their permeability across physiological barriers. Thus, they bring about substantial changes in drug biodistribution. They also minimize the toxicity and other adverse side effects associated with some important drugs. Moreover, the pharmaceutical polymeric micelles chosen for effective drug delivery also have a high drug-loading capacity, a controlled release profile for the incorporated drug and good compatibility between the core-forming polymeric block and the incorporated drug. Following intravenous administration, they also circulate in the blood for a longer time because of their smaller size and hydrophobic shell that minimize their uptake by RES. In addition, micelles can be made target specific by the chemical attachment of a targeting moiety to their surface; the local release of the loaded drug in the target organ and its efficacy can be improved extensively. On the other hand, because it is in a micellar form en route to the target organ or tissue, the drug is well protected from possible inactivation due to biological surroundings, and it also does not provoke undesirable side effects on non-targeted organs and tissues. Among other factors influencing the efficacy of drug loading into the micelle is the size of both core-forming and corona-forming blocks.[118] Researchers have seen that a narrow size-range of micelles act as a crucial factor in determining their transport and retention in tissues showing EPR effect.

Due to such unique properties, polymeric micelles have been well demonstrated as effective drug carriers. Drugs, such as diazepam, indomethacin,[119,120] adriamycin,[121] anthracycline antibiotics[122] and polynucleotides[123] were effectively solubilized by polymeric micelles and demonstrated superior properties and lower toxicity. In comparison with free drugs, adriamycin in polymeric micelles was shown to be much more efficient in experimental treatment of murine solid tumor colon adenocarcinoma.[124] PEG-b-poly (caprolactone) copolymer micelles were also successfully used as delivery vehicles for dihydrotestosterone.[125] A system in which doxorubicin (DOX) is conjugated to PEG-poly (α, β-aspartic acid) block copolymer [PEG-PAsp (DOX)], formed micelles with both chemically bound and physically entrapped DOX in the core. These drug-loaded micelles achieved prolonged circulation in the blood compartment due to reduced uptake into the RES and accumulated notably in the solid tumor through EPR effect, leading to complete tumor regression mainly by sustained release of physically entrapped DOX from tumor-localized micelles.[126] Studies also show that PEG-phosphatidylethanolamine (PEG-PE) micelles can efficiently incorporate a number of sparingly soluble substances like paclitaxel, tamoxifen, campothesin, porphyrine and vitamins.[127] Furthermore, targeted micelle formulations from PEG_{750}-PE, PEG_{2000}-PE and PEG_{5000}-PE conjugates demonstrated much higher accumulation in tumors in comparison with nontarget

tissues (muscle) in experimental Lewis lung carcinoma cells in mice.[128] Some data also indicate spontaneous targeting of PEG-PE-based micelles into damaged heart areas in rabbits with experimental myocardial infarction.[129] Micelles made up of thermo- or pH-sensitive components such as poly (N-isopropylacrylamide) and its copolymers with poly (D, L-lactide) can disintegrate under conditions of increased temperature or decreased pH values (generally associated with many pathological processes in various tissues and organs) and release the micelle-incorporated drug in the target areas.[130] In addition, pH-responsive polymeric micelles loaded with phtalocyanine also seem to be promising carriers for photodynamic cancer therapy,[131] and DOX-loaded polymeric micelles containing acid-cleavable linkages provided an enhanced intracellular drug delivery into tumor cells and thus higher efficiency.[132]

The drug-delivery potential of polymeric micelles can be further enhanced by attaching specific ligands such as sugar moieties, transferrin (Tf) or folate, etc., to the water-exposed termini of the hydrophilic blocks. Such micelles are highly effective, because various target cells, especially cancer cells, overexpress appropriate receptors like Tf and folate receptors on their surface. Tf-modified micelles based on PEG and poly (ethyleneimine) sized between 70 and 100 nm are expected to target tumors with overexpressed Tf receptors.[133] Poly (L-histidine)/PEG and poly (L-lactic acid)/PEG block copolymer micelles carrying folate residues on their surface were shown to be efficient for the delivery of adriamycin to tumor cells in vitro.[134] Side-by-side immunomicelles prepared by covalently attaching antibodies to a surfactant or polymeric micelles[135] have also shown great potential in targeted delivery. Antibodies attached to the micelle corona preserve their specific binding ability, and immunomicelles specifically recognize their target substrates. Certain nonpathogenic monoclonal antinuclear autoantibodies with nucleosome-restricted specificity, like 2C5 (mAb 2C) that can bind a broad variety of cancer cells, increased their efficiency in targeting tumor cells when attached to micelles. Loaded with the poorly soluble anticancer drug Tx, mAb 2C5 immunomicelles demonstrate significantly increased cytotoxicity toward tumor cells in vitro and in vivo.[115] Moreover, it is also seen that after being entrapped in the polymeric micelles, the antifungal activity of amphotericin B is enhanced and its hemolytic activity also is reduced.[136] RGD peptides that target integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, markers of angiogenic endothelial cells, can also be coupled to the surface of micelles for the delivery of combretastatin A4 to tumor vasculature in vitro. Studies demonstrated superior antiproliferative efficacy as well as enhanced intracellular delivery over non-targeted micelles.[137] Cancer cell-specific phage proteins identified from phage display peptide libraries can serve as targeting ligands for polymeric micelle-based pharmaceutical preparations was recently demonstrated by Wang et al through the preparation of paclitaxel-loaded polymeric micelles modified with MCF-7 cell-specific phage protein. These targeted paclitaxel-loaded phage micelles demonstrated a significantly higher cytotoxicity toward target MCF-7 cells than free drug or non-targeted micelle formulations.[138] Thus, widespread use of polymeric micelles is expected in the field of drug delivery.

Dendrimers

Dendrimers derive their name from the Greek word *dendra*, meaning reminiscent of a tree. They are polymeric molecules composed of multiple perfectly branched monomers that emanate radially from a central core. Though many biological applications use dendrimers based on polymers, such as polyamidoamines (PAMAMs), polyamines, polyamides (polypeptides), poly (aryl ethers), polyesters, carbohydrates and DNA, in most cases PAMAM dendrimers are used.[139,140] They consist of three critical architectural domains: (i) the multivalent surface, containing a larger number of potentially reactive sites, (ii) the interior shells (i.e., branch cell layers defined by dendrons) surrounding the core, and (iii) the core to which the dendrons are attached. These three domains can be tailored to serve various purposes, such as dendritic sensors, drug and gene carriers, or themselves as drugs. In dendrimers, the number of branch points encountered moving upward from the core of the dendrons to the periphery is defined as their generation number (G-1, G-2, G-3, G-4). The generation number of the dendrimers can also be altered to enhance their drug-loading capacity. In addition, the high density of exo-presented surface functionalities makes the dendritic surface well suited as a nano-scaffold where the close proximity of functional groups is important or for receptor-mediated targeting purposes. On the other hand, the interior is well suited for host–guest interaction and encapsulation of guest molecules. Furthermore, the end groups of dendrimers can also be modulated to alter dendrimers' solubility; for instance, the hydrophilic end groups can make a dendrimer with a hydrophobic core water soluble, whereas hydrophobic peripheral moieties can make a dendrimer with a hydrophilic interior soluble in oil.[141] In addition, the controlled degradation of the dendrimers can be achieved by the judicious choice of their chemistry and thus they can be made biocompatible.

Due to the above-mentioned qualities, dendrimers can be harnessed as effective carriers of many pharmaceuticals. One of the earliest examples of antitumor drug delivery using dendrimers was achieved by complexing the anticancer drug cisplatin to the surface groups of a G-4 carboxylate-terminated PAMAM dendrimer. These conjugates exhibited slower release, higher accumulation in solid tumors and lower toxicity in comparison with free cisplatin.[142] PAMAM dendrimers have also been used as antitumor-targeted carriers of MTX. Studies showed that after IV administration in mice bearing subcutaneous cervical carcinoma KB tumors, radiolabled or flourescently labeled folate dendrimers accumulated and were taken up intracellularly by human KB tumors overexpressing the folic acid receptor. The concentration of the targeted dendrimer was also 5 – 10 times higher than the control dendrimer lacking the folate ligand. Thus a treatment of these mice with 15 biweekly IV injections of the MTX-folate-fluorescein-modified dendrimers significantly reduced the rate of tumor growth relative to the saline-treated mice.[143] In another study, the anticancer drug 5-fluorouracil encapsulated into PAMAM dendrimers with carboxymethyl PEG revealed reasonable drug loading and reduced release rate and hemolytic toxicity compared to the non-PEGylated dendrimer.[144] The dendrimers have also been proved to act as insoluble supports for the delivery of therapeutic molecules. Grinstaff et al have

Table 5
Several nanotechnology-based products that are being tested or have been approved

Product	Type of Nanomaterial	Indicator	Phase	Advantages	Company
Doxil	PEGylated liposome	Ovarian cancer and multiple myeloma	On Market	Enhanced circulation time and is up to six times more effective than free DOX.	Janssen
Abraxane	Albumin NPs	Lung Cancer Breast Cancer	On Market	Enhanced cytotoxicity, shorter infusion time, low dose required	Celgene Corporation/ Abraxis Biosciences
Aurimmune (CYT-6091)	AuNPs coupled to TNF and PEG-Thiol	Solid Tumors	Phase II	Selectively destroys cancer cells without harming healthy tissues	CytImmune Sciences
AuroShell	Gold-coated silica NPs	Solid Tumors	Phase I	Highly selective and rapid tumor destruction with minimal damage to surrounding healthy tissues	Nanospectra Biosciences
Combidex	Iron oxide NPs	Tumor Imaging	NDA Filed	Efficient for the detection of metastatic lymph nodes in various cancers	Advanced Magnetics
Cyclosert	Cyclodextrin NPs	Metastatic solid tumor	IND Filed	Very effective in preventing tumor progression	Insert therapeutics
ING N-401	Liposome	Metastatic lung cancer	Phase I	Suppresses tumor growth and inhibits metastasis of lung cancer	Introgen
MRX-952	Formulation of irinolecan metabolite	Oncology	Preclinical		ImaRx therapeutics
Nanoxel	Nanoparticlulate delivery system for paclitaxel	Breast Cancer Ovarian Cancer	On market	Cremophor free water-soluble formulation. Greater efficacy and decreased cytotoxicity	Dabur Pharma
TNT AntiEpCAM	Polymer-coated iron oxide	Solid Tumors	Preclinical	Provides targeted therapy. Selectively kills EpCAM positive cells	Triton BioSystems
Verigene platform	DNA functionalized AuNPs	Diagnostics	On market	AuNPs enable efficient diagnosis for methicillin-resistant *Staphylococcus aureus*	Nanosphere Triton BioSystems

Abbreviations: NDA, New Drug Application; IND, Investigational New Drug.
Source: http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

shown that the dendrimers' high functional-group density and low solution viscosities make them useful as injectable sealants for corneal wounds. In their work Grinstaff et al functionalized the peripheries of the biodegradable polyester dendrimers with reactive groups that can cross-link and form an insoluble hydrogel matrix upon activation. Maximum intraocular pressures before rupture in eyes sealed with the dendrimers were comparable with those attained by more common and labor-intensive suturing method.[145,146] It was also found that ibuprofen, when encapsulated in PAMAM dendrimers, is successfully transported to lung epithelial carcinoma cells.[147] The studies with Caco-2 cell lines indicated that the low generation PAMAM dendrimers cross cell membranes presumably through a combination of two processes: paracellular transport and absorptive endocytosis; the cell efflux systems have a minor effect.[148] Dendrimers have also revealed their potential in transdermal drug delivery. It has been shown that PAMAM dendrimers enhanced the bioavailability of indomethacin in transdermal delivery applications.[148] Dendrimers can also be used as ocular drug-delivery vehicles. A complex of puerarin and poly(amidoamine) (PAMAM) dendrimers was prepared that produced longer ocular residence times in rabbits in comparison with puerarin eye drops, thus representing a potential ocular drug-delivery system to improve the efficacy of drug treatment.[149] A poly (l-glutamic acid) dendrimer-based drug-delivery system with both pH-sensitive and targeting functions has also been reported by Yuan et al that shows greater antitumor activity and internalization and are promising vectors for fabricating smart and targeting drug-delivery systems.[150]

Currently, researchers are also investigating using dendrimers as potential gene-delivery vehicles because dendrimers form compact polycations under physiological conditions. Different functionalized PAMAM dendrimers, poly (propylene imine) dendrimers and partially hydrolyzed PAMAM dendrimers have been effectively used as DNA-delivery systems.[151] PAMAM dendrimers functionalized with α-cyclodextrin showed luciferase gene expression about 100-fold higher than for unfunctionalized PAMAM or for noncovalent mixtures of PAMAM and α-cyclodextrin.[152] PEG functionalization of G-5 PAMAM dendrimers also produced a 20-fold increase in transfection efficiency using plasmid DNA coding for a reporter protein β-galactosidase relative to partially degraded PAMAM dendrimers.[153] Furthermore, advanced and sophisticated drug-delivery systems can also be prepared by using dendrimers. One study depicts that a "bow-tie" structure can be prepared by covalently conjugating 2 polyester dendrons, where 1 dendron provides multiple functional handles for the attachment of the drug molecules, and the other dendron is used to attach solubilizing poly(ethylene oxide) (PEO) chains. By varying the generations of dendrons and the mass of the PEO chains, molecular weight, architecture and drug-loading capacity can be readily controlled.[154] Another study employed the G-4 PAMAM dendrimers to construct dendrimer/poly (styrenesulfonate) (PSS) microcapsules following a layer-by-layer deposition protocol of both constituents around a removable melamine formaldehyde colloidal core. These PAMAM/PSS capsules can allow the selective encapsulation of drug into the capsule core and into the dendrimers, which are localized within the shell of the capsule, thus providing a dual release system of either two different drugs (i.e., drug cocktail) or of one drug released following two different time protocols (i.e., fast and sustained release).[155] Although small interfering RNA (siRNA) treatment holds great

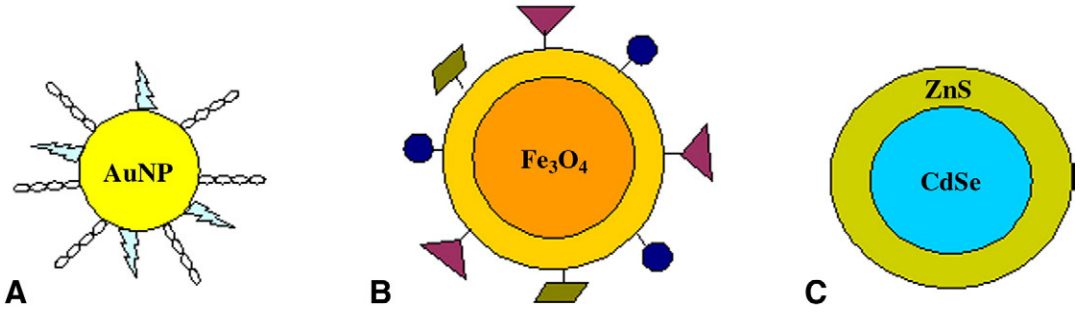

Figure 6. Schematic illustration of **(A)** functionalized AuNPs **(B)** magnetic NPs with ferrite core covered by stabilization shells, e.g., SiO_2 **(C)** QDs that can be used for diagnostics and imaging.

promise for the treatment of cancers, the field has been hindered by the availability of suitable delivery vehicles. Cationic dendrimers can also greatly improve the stability of siRNA, its intracellular trafficking, its silencing efficacy and its accumulation in the tumor environment owing to the EPR effect. Significant gene silencing was accomplished in vivo within 24 hours of treatment with luciferase siRNA-nanocarrier polyplexes developed by Ofek et al. These polymerized polyglycerol-based dendrimer core shell structures were able to deliver siRNA to tumors in vivo and showed very low levels of toxicity because no significant weight loss was observed after IV administration of the polyplexes.[156] In another study, the potential of dendrosomes for the delivery of siRNA targeting E6 and E7 proteins of cervical cancer cells was explored in vitro and their results suggested that dendrosomes hold potential for the delivery of siRNA and thus could be a suitable targeting strategy for applications in vivo.[157] Dendrimers have a great potential to be used as effective drug-delivery vehicles.

Clinically used NP dispersion formulations

Although this is a relatively new field, various formulations are in clinical trials or have been successfully marketed. These NPs offer specific advantages over the conventional therapeutics, such as low dosing, specific targeting and enhanced cytotoxicity. A list of the NPs in the market or clinical trails are listed in Table 5.

Toxicities related to nanoformulation

The benefits of nanoparticle drug-delivery systems are manifold. NPs are highly stable, encapsulate both hydrophilic and hydrophobic substances, and are highly compatibile with diverse administration routes (oral, inhalation, etc.). NPs provide sustained release of drug from the polymeric matrix. These properties of NPs support in improvements in drug bioavailability values and dosing frequency and could resolve the common problem of noncompliance with prescribed therapy. The use of NPs as drug carriers may also reduce the toxicity of the incorporated drug. However, a number of different classes of NPs with different physico-chemical properties account for the adverse biological responses.[158,159] To use the potential of nanotechnology in nanomedicine, full attention must be given to safety and toxicological issues. Specific importance should be accorded to the toxicity of the empty non-drug-loaded NPs. In case of slowly degradable or nondegradable NPs used for drug delivery may show persistence and accumulation at the site of the drug delivery, ultimately resulting in chronic inflammatory response.[160] NPs are attributed qualitatively different physico-chemical characteristics from bulky materials, which may lead to changes in body distribution, passage across the BBB, and triggering of blood coagulation pathways.[160] Cationic NPs including Au and polystyrene have been shown to cause hemolysis and blood clotting, and usually anionic particles are rather non-toxic.[94] Recent studies with carbon-derived nanomaterials showed that platelet aggregation was induced by both single and multiwalled carbon nanotubes.[161] Various studies with fullerenes have investigated the ecotoxicity of these important building blocks in nanomaterials.[162,163] Choi et al have demonstrated that the nonmodified cadmium telluride QDs induced lipid peroxidation in the cells.[164] Cho et al showed naked QDs to be cytotoxic by induction of reactive oxygen species (ROS), resulting in damage to plasma membranes, mitochondria and nucleus.[165] For silica NPs researchers found an augment in toxicity both at increasing doses and at increasing exposure times. Silicon oxide exposure resulted in an increased ROS levels and reduced glutathione levels indicating an increased in oxidative stress.[166] Chang et al have reported that silica NPs are toxic at high dosages as revealed by reduction in cell viability and by lactate dehydrogenase release from the cells indicating membrane damage.[167] NPs could also cause mitochondrial damage, uptake through olfactory epithelium, platelet aggregation and cardiovascular effects. These effects require a novel way of handling the toxicology of NPs.[168]

Outlook and future challenges

Though nanotechnology has grown rapidly and NPs are entities that can revolutionize therapy, imaging and early diagnosis of various diseases, some inherent problems need to be addressed. While developing therapeutics, attention should be given to toxicity. Coated or uncoated NPs have a tendency (in varying amounts) to accumulate in the liver. Therefore, a detailed mechanism for exclusion from the body needs to be properly addressed. Moreover, while formulating NPs, an important aspect is to minimize the batch-to-batch variations; the synthetic

yield and the drug-loading effectiveness must also be boosted to warrant practical utility of the NPs. In addition, the mechanism of endocytosis and degradation pathways needs to be addressed because these are still poorly understood, despite their primary importance for clinical transition. Furthermore, there is an urgent need for the development of safety guidelines by the government regarding the environmental effects and the potential effects on the health of people manufacturing the NPs. Despite these concerns, the most exciting prospect of nanocarriers is the near limitless possibilities for treatment strategies.

The versatility of formulation, colloidal size, biocompatibility and sustained-release properties of NPs have already been accepted with growing interest for a wide range of applications. However, the recent strategy gaining widespread interest and attention is that of "functionalized NPs." Methods are being devised to tailor the surface characteristics of NPs to achieve specific ligand-mediated targeting of therapeutic and imaging agents. Optimization of these techniques, coupled with the considerable progress made in the field of NP characterization and a better understanding NPs' in vivo behavior has raised hopes for the successful development of commercial products based on targeted NPs for use in therapy and imaging (Figure 6).

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