
5 Multifunctional Nanoparticles for Cancer Therapy

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5.1 INTRODUCTION

The use of nanoparticles in cancer therapy is attractive for several reasons: they exhibit unique pharmacokinetics, including minimal renal filtration; they have high surface-to-volume ratios enabling modification with various surface functional groups that home, internalize, or stabilize; and they may be constructed from a wide range of materials used to encapsulate or solubilize therapeutic agents for drug delivery or to provide unique optical, magnetic, and electrical properties for imaging and remote actuation. The topology of a nanoparticle—core, coating, and surface functional groups—makes it particularly amenable to modular design, whereby features and functional moieties may be interchanged or combined. Although many functionalities of nanoparticles have been demonstrated, including some clinically approved drug formulations and imaging agents,^{3,8} the consolidation of these into multifunctional nanoparticles capable of targeting, imaging, and delivering therapeutics is an exciting area of research that holds great promise for cancer therapy in the future.

Figure 5.1¹ schematically depicts a hypothetical multifunctional particle that has been engineered to include many features such as the ability to target tumors, evade uptake by the reticuloendothelial system (RES), protect therapeutics that can be released on demand, act as sensors of tumor responsiveness, and provide image contrast to visualize sites of disease and monitor disease progression. Some of these features, such as targeting, leverage biological machinery. Others are derived synthetically and enable external probing or manipulation that is otherwise not feasible in biological systems. In this chapter, we review both bio-inspired and synthetic nanoparticle functionalities that have been used in cancer therapy and address both current efforts and future opportunities to combine these into multifunctional devices.

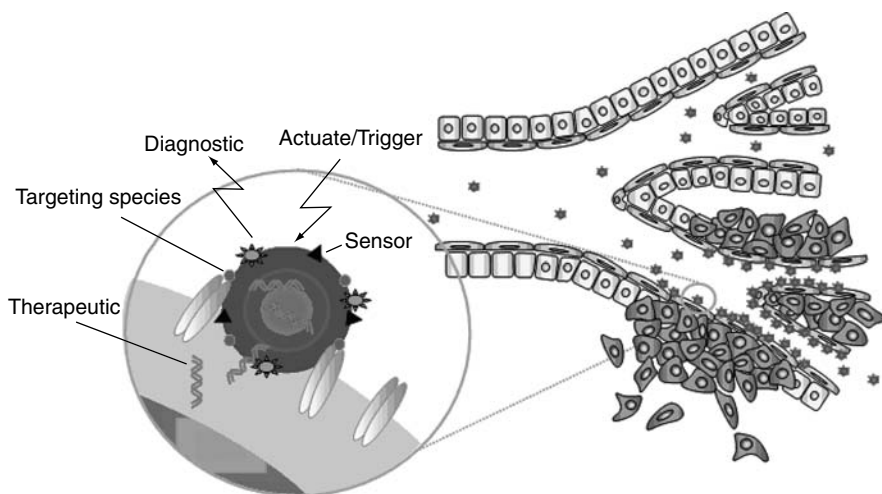


FIGURE 5.1 Schematic depiction of a multifunctional nanoparticle. A hypothetical nanoparticle targets the tumor, senses and reports molecular signatures, and delivers a therapeutic in response to an external or biological trigger. (From Ruoslahti, E., *Cancer Cell*, 2, 97–98, 2002.)

5.2 MODULAR FUNCTIONALITIES AT THE BIOSYNTHETIC INTERFACE

5.2.1 TARGETING

The ability to physically target diseased cells to receive therapeutics while avoiding residual uptake in other tissues has long been a goal in cancer therapy.^{9–12} The homing of stem cells to a tissue niche, or the susceptibility of one cell over another to viral infection, demonstrates that biomolecular recognition can be used to direct species to specific extracellular and intracellular sites. The microenvironment of the tumor including cell-surface markers, extracellular matrix, soluble factors, and proteases, as well as the tumor's unique architecture and transport properties, may be exploited for targeting.^{13–17}

Both passive and active targeting have been utilized for nanoparticle delivery. Passive targeting relies upon the unique pharmacokinetics of nanoparticles including minimal renal clearance and enhanced permeability and retention (EPR) through the porous angiogenic vessels in the tumor.^{18,19} Surface attachment of polymers such as poly(ethylene glycol) (PEG) and poly(ethylene oxide) (PEO) enables nanoparticles to avoid uptake by mononuclear phagocytes in the liver, spleen, and lymph nodes, thereby improving accumulation in the tumor.^{20–22} Active targeting relies on ligand-directed binding of nanoparticles to receptors expressed in the tumor. Binding of ligands to the vasculature can occur immediately, as it is directly accessible to nanoparticles circulating in the blood. Over longer time periods, particles extravasate into the tissues where receptors expressed on cancer cells and in the interstitium may be used for localization.^{23–25}

Many candidate tumor markers have been described, some of which bind known ligands such as arginine–glycine–aspartic acid (RGD)-binding $\nu\beta_3$ and $\nu\beta_5$ integrins expressed on the surface of angiogenic blood vessels, and folic acid-binding receptors on the surface of cancer cells. These and others have been attached to the surface of various nanoparticle cores to deliver them to tumors.^{17,26–28} Monoclonal antibodies have also been used extensively for targeting. These can be isolated with high affinity for tumor markers and are useful for targeting receptors of unknown or low affinity ligands.^{29,30} Novel screens for discovering tumor homing ligands have been developed using phage and bacterial display as well as libraries of aptamers, peptides, polymers, and small molecules.^{31,32} These techniques may be used to isolate targeting ligands, even when their target

receptor is unknown. For example, the 34 amino acid, cationic peptide F3, which has been used to deliver quantum dots to tumor endothelium, was uncovered initially by a blind-page display screen in a breast cancer xenograft model and later found to bind cell surface nucleolin expressed on tumor endothelium and cancer cells.^{6,33,34}

Although extracellular targeting to the tumor is sufficient for many modes of imaging and drug delivery, intracellular delivery of nanoparticles into the cytosol is essential for some applications. For example, nanoparticles carrying membrane-impermeable cargo that perform their function in the cytosol, such as siRNA, antisense DNA, peptides, and other drugs, are minimally effective if delivered extracellularly or sequestered in the endosome.³⁵ Protein and peptide motifs capable of translocating nanoparticles into the cytoplasm have been borrowed from mechanisms of viral transfection. Two important classes of translocating domains include polycationic sequences and membrane fusion domains. Attaching the short polycationic sequence of HIV's TAT protein, amino acid residues 48–57, to a nanoparticle facilitates its adsorption on a cell surface and subsequent internalization into the cell.^{36,37} This peptide has been used to internalize dextran coated iron-oxide nanoparticles into T-cells *in vitro*, which were subsequently used to monitor T-cell trafficking in tumors with MRI.³⁸ Use of this peptide for intracellular delivery *in vivo* is limited by the adverse effect that polycationic sequences have on nanoparticle circulation time and RES uptake.³⁹ The amphiphilic domain derived from the N-terminus of the influenza protein hemagglutinin (HA2) is a membrane fusion peptide that destabilizes the endosome at low pH and facilitates viral escape into the cytosol.⁴⁰ Variations of this peptide with improved infectivity have also been synthesized.⁴¹ Influenza-derived peptides have been used to enhance the delivery of liposomes as well as 100 nm poly-L-lysine particles. Although the peptide modification of these particles improves endosomal escape over unmodified particles, the transfection efficiency still remains well below that of intact viruses.^{42,43}

Another level of targeting can occur after translocation of nanoparticles into the cytosol to direct nanoparticles to specific sub-cellular structures. Using peptide localization sequences, fluorescent quantum dots have been targeted to the nucleus and the mitochondria (Figure 5.2).² Several other localization sequences exist and could be used to traffic nanoparticles to the endoplasmic reticulum, golgi apparatus, or peroxisomes. Although work in this area has been focused on organelle labeling, the potential for delivering therapeutic nanoparticles to sub-cellular structures is possible. Such nanoparticles could sense sub-cellular aspects of disease or specifically intervene for more potent treatment or eradication of cancer cells (i.e., free-radical-mediated mitochondrial damage to induce apoptosis).

5.2.2 IMAGING AGENTS

Imaging cancer is crucial for guiding decisions about treatment and for monitoring the efficacy of administered therapies. The use of nanoparticles for image contrast and enhancement has enabled improvements in cancer imaging by conventional modalities, such as magnetic resonance imaging (MRI) and ultrasound, and has also established new techniques such as optical-based imaging for cancer detection.^{39,44,45} Targeted imaging agents that can identify specific biomarkers have the potential to improve detection, classification, and treatment of cancer with minimal invasiveness and reduced costs.

The use of nanoparticles in cancer imaging has already demonstrated clinical efficacy in detecting liver cancer and staging lymph node metastasis noninvasively.^{3,46} Superparamagnetic iron-oxide nanoparticles disrupt local magnetic field gradients in tissues, causing a detectable signal void in MRI. Dextran coated iron-oxide nanoparticles administered intravenously get phagocytosed by normal macrophages of the liver and lymph and the failure of these tissues to darken after iron-oxide administration identifies invading cancer cells. Directly targeting these magnetic nanoparticles to cancer cells has also been demonstrated. For example, herceptin mAb and folic acid on the surface of iron-oxide nanoparticles enable MRI-based molecular imaging of their respective targets

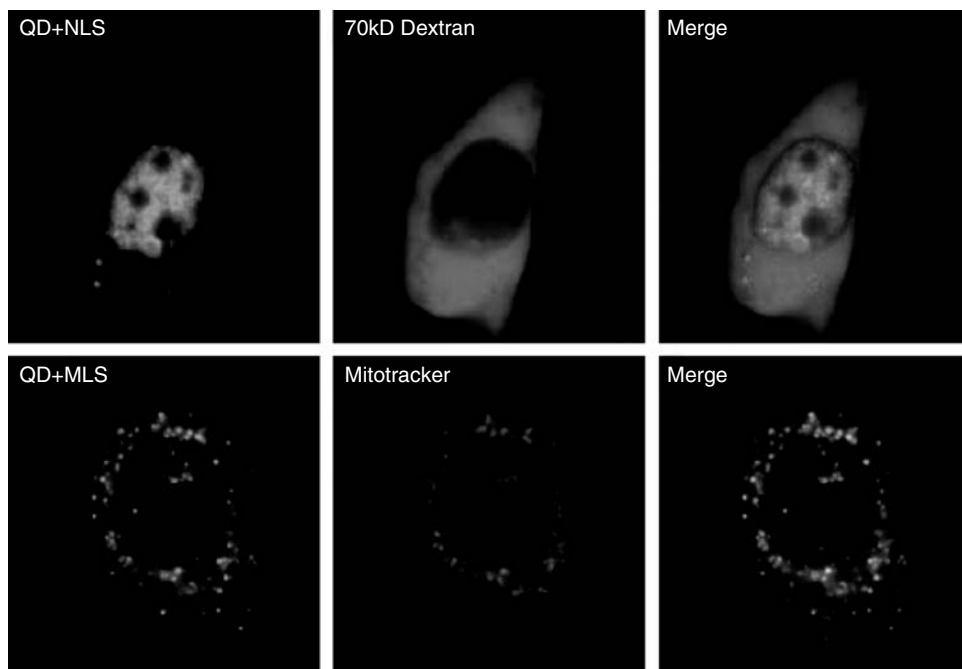


FIGURE 5.2 (See color insert following page 522.) Labeling of intracellular targets with peptide labeled quantum dots. Quantum dots (QD) modified with PEG and a nuclear localization sequence (NLS) or a mitochondrial localization sequence (MLS) were shown to target the nucleus or mitochondria of cells respectively. Seventy kilo Dalton PEG distributed in the cytoplasm contrasts nuclear localization while MitoTracker colocalizes with mitochondrial localization. (From Derfus, A. M., Chan, W. C. W., and Bhatia, S. N., *Advanced Materials*, 16, 961, 2004.)

in tumors.^{47,48} Other nanoparticle cores including dendrimers, micelles, and liposomes modified with paramagnetic gadolinium have also been used for tumor targeted MRI contrast.^{48–50}

Gold nanoshells offer a promising alternative to MRI probes by providing contrast for optical imaging.⁴⁵ These nanoparticles are constructed from a dielectric core (silicon) and a metallic conducting shell (gold). By varying the dimension of the core and shell, the plasmon resonance of these particles can be engineered to either absorb or scatter wavelengths of light, from UV to infrared. Particles that are tailored to scatter light in the near-infrared, where tissues have minimal absorbance, have been used to enhance imaging modalities such as reflectance confocal microscopy and optical coherence tomography (OCT).⁵¹ Although the penetration of optical techniques does not approach that of CT or MRI, imaging features is possible at depths of a few centimeters. Gold colloids have also been used for optical contrast, but these lack the inherent tunability of nanoshells. The conjugation of optical contrast agents to antibodies has been used for the molecular imaging of the EGFR receptor on early cervical precancers and for Her2+ breast carcinoma cells in mice.^{52,53}

Fluorescent nanoparticles offer another useful tool to enhance optical detection. These probes are identified easily in microscopy and are useful for tracking the biodistribution of nanoparticles in experimental models. Fluorescent semiconductor nanocrystals, quantum dots, have been used to show ligand-mediated nanoparticle targeting to distinct features in the tumor.⁶ Three different phage display-derived peptides were used to specifically target these nanocrystals to tumor blood vessels, tumor lymphatics, or lung endothelium (Figure 5.3). Functionalization of these nanoparticles with PEG eliminated detectable accumulation in RES organs, including the liver (Figure 5.4). Quantum dots have a distinct advantage over conventional fluorophores because

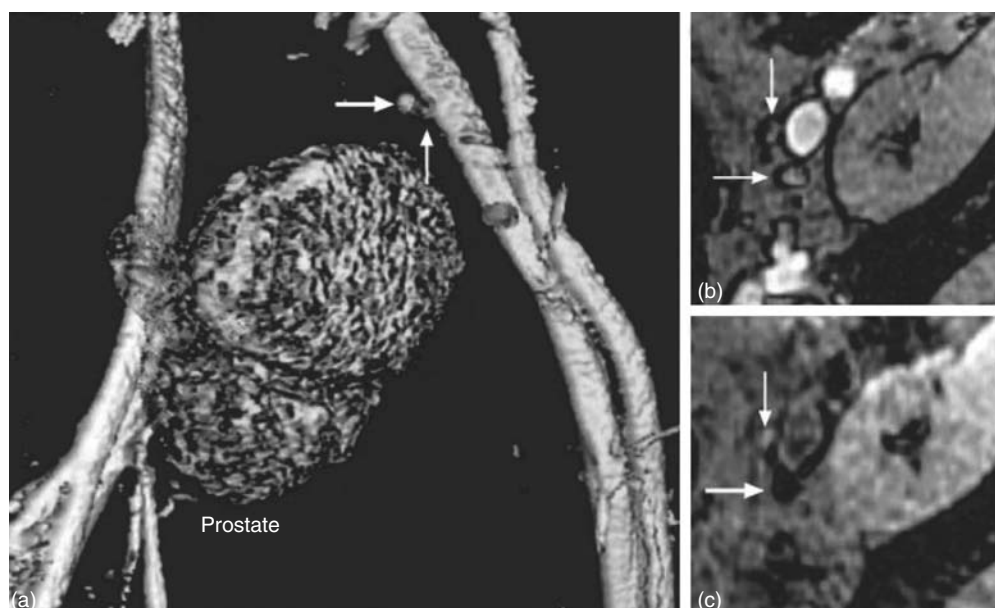


FIGURE 5.3 (See color insert following page 522.) Noninvasive detection of lymph node metastasis with iron-oxide nanoparticles and MRI. (a) A three-dimensional reconstruction using nanoparticle-enhanced MRI of the prostate. Metastatic lymph nodes are in red and normal nodes are in green. (b) Conventional MRI image shows similar signal intensity from two adjacent nodes. (c) Nanoparticle-enhanced MRI shows a decreased signal in the normal node from macrophage uptake (thick arrow), but not in the metastatic node (thin arrow). (From Harisinghani, M. G. and Weissleder, R., *Plos Medicine*, 1, 202–209, 2004.)

of their size-tunable excitation and emission profiles, narrow bandwidths, and high photo-stability.^{54,55} Using nanocrystals that fluoresce in the near-infrared could extend their utility to clinical settings,⁵⁶ though a key limitation has been their potential toxicity because they are formulated from heavy metals.⁵⁷ Efforts to make these of nontoxic materials are ongoing (Figure 5.4). Alternative fluorescent nanoparticle probes have been developed including fluorescently tagged dendrimers and fluorophore-embedded silica nanoparticles.^{58–60}

Nanoparticle formulations that provide contrast for other imaging modalities including ultrasound and CT have been described. Perfluorocarbon emulsion nanoparticles composed of lipid-encapsulated perfluorocarbon liquid, about 250 nm diameter, are effective in giving echo contrast.⁶¹ Air-entrapping liposomes formulated from freeze-drying techniques have also been developed to give ultrasound contrast.^{62,63} These agents passively distribute in RES organs and areas of angiogenesis enabling enhanced imaging of these features. Bismuth sulfide nanoparticles can be used as contrast agents for CT imaging, giving blood pool contrast similar to that of iodine but at lower concentrations. These can also be used to image lymph nodes after phagocytic uptake.⁶⁴ As with other imaging modalities, the attachment of appropriate ligands to nanoparticle-based CT and ultrasound contrast agents could be used for molecular imaging.

An interesting extension of the imaging agents described above is their combination into multimodal imaging nanoparticles. The combination of fluorescence and magnetic properties within a single particle has been used for dual optical and MRI imaging. Fluorescence is used to determine the localization of targeted imaging agents down to specific micro-structures inside and outside cells and magnetic domains provide three-dimensional whole-body imaging capabilities with MRI.^{59,65} These magneto-fluorescent nanoparticles could be effective for image guided surgeries where MRI is used to locate cancer in the body and fluorescence is used to more precisely

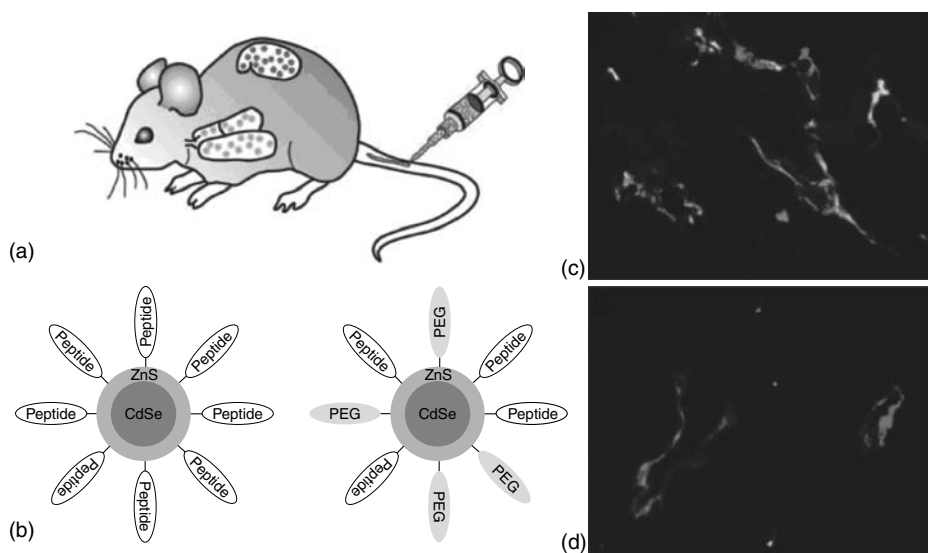


FIGURE 5.4 (See color insert following page 522.) Targeting quantum dots (QDs) to site-specific endothelium with phage display-derived peptides. (a) Schematic representation of co-injected red and green quantum dots that home to tumor and lung vasculature respectively after intravenous injection. (b) Schematic representation of peptide-coated QDs and peptide-coated PEG-QDs. (c) QDs labeled with the tumor endothelium homing peptide F3 co-localize with a blood vessel marker. (d) QDs labeled with the tumor lymphatic homing peptide Lyp-1 highlight the endothelium but do not colocalize with a blood vessel marker. (From Akerman, M. E., Chan, W. C. W., Laakkonen, P., Bhatia, S. N., and Ruoslahti, E., *Proceedings of the National Academy of Sciences of the United States of America*, 99, 12617–12621, 2002.)

delineate tumor borders during resection. Other dual-imaging probes have been described including: perfluorocarbon emulsions tagged with gadolinium for combined ultrasound and MRI.⁶⁶

5.2.3 SENSING

Functionalities that undergo chemical alterations in response to enzymatic activity or other properties such as pH or oxygen could be used as sensors to report information about the status of the tumor or efficacy of treatment. Many nanoparticle-based sensors that respond to biological triggers including proteases, DNases, proteins, peroxidase, pH, and others have been demonstrated *in vitro*.^{67–72} These generally rely on assembly or disassembly of inorganic nanocrystals including: gold nanoparticles, nanoshells, or nanorods, which undergo a shift in their plasmon resonance when aggregated; iron-oxide nanoparticles have enhanced T2 relaxivity when clustered; and fluorescent quencher-based nanoparticle systems that dequench after triggered release.

A system using cleavable polymeric shielding of self-assembling nanoparticles has been proposed as a mechanism for translating nanoparticle-based enzyme sensors to *in vivo* use (Figure 5.5).⁷³ Self-assembling, complementary iron-oxide nanoparticles are rendered latent with PEG polymers linked to the nanoparticle surface by protease-cleavable substrates that serve to both inhibit assembly and stabilize the particles in serum. Upon proteolytic removal of PEG polymers by MMP-2 expressing cancer cells, nanoparticles assemble and acquire amplified magnetic properties that can be detected with MRI. In the future, similar to thrombin-driven self-assembly of fibrin and platelets at sites of endothelial injury, this system may allow the hyper-active proteolytic processes of cancers to drive the self-assembly of nanoparticles in regions of cancer angiogenesis, invasion, and metastasis *in vivo*. Due to its modular design, this system can easily be modified for a number of detection schemes by substituting the complementary binding pairs, cleavable substrates

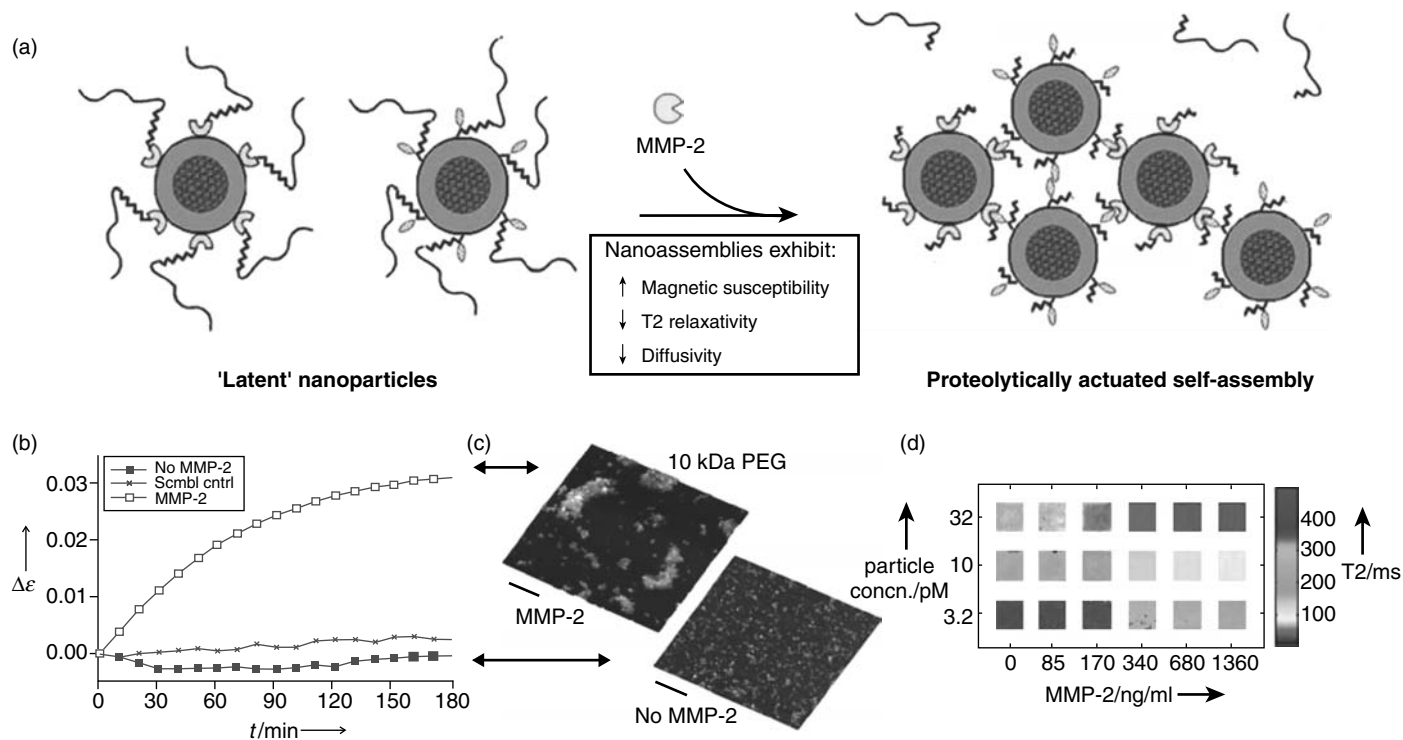


FIGURE 5.5 (See color insert following page 522.) Protease-triggered self-assembling nanoparticles with polymer-shielded coatings. (a) Schematic representation of nanoparticles that self-assemble after protease-mediated cleavage of PEG chains reveals complementary moieties (neutravidin and biotin). (b) Iron-oxide nanoparticles with cleavable linkers assemble in the presence of MMP-2, as measured by changes in their light extinction, while particles with noncleavable scrambled peptides do not. (c) Atomic force micrographs of particles incubated with MMP-2 show detectable aggregation (scale bars are 500 nm). (d) T2 maps of particles in solution using a 4.7 T MRI demonstrate enhanced T2 relaxivity for increasing concentrations of MMP-2.

(e.g., glycans, lipids, oligonucleotides), or multivalent nanoparticle cores (e.g., gold, quantum dot, dendrimer).

5.2.4 THERAPEUTIC PAYLOADS

The use of nanoparticulate drug carriers can address many critical challenges in drug delivery including: improving drug solubility and stability; extending drug half-lives in the blood; reducing adverse effects in nontarget organs; and concentrating drugs at the disease site.⁷⁴ Drugs may be dispersed in a matrix, encapsulated in a vesicle, dissolved in a hydrophobic core, or attached to the surface of a nanoparticle. Several nanoparticle-based drug delivery systems including liposomes, polymeric nanoparticles, dendrimers, ceramic based carriers, micelles, and others have been used to carry small molecule, peptide, and oligonucleotide therapeutic agents.^{24,75} Many promising anti-cancer drugs fail to make it to the clinic because of poor solubility or high collateral toxicity at therapeutic levels, thus motivating the need for these carriers in cancer therapy.

Liposomes have been the most extensively utilized nanoparticle-based carriers for delivering anti-cancer drugs. First described decades ago, these submicron-sized carriers consist of amphiphilic lipids assembled to form vesicles that can encapsulate drugs.⁷⁶ Liposome-encapsulated doxorubicin is a clinically approved nanoparticle formulation used for chemotherapy.⁷⁷ The surface of this nanocarrier is PEGylated to reduce rapid uptake by phagocytic cells and extend the drug circulation time for better therapeutic efficacy. Several other liposome-encapsulated chemotherapeutic drugs have been described, with many in clinical trials.⁷⁸ Active targeting of these liposomes through the attachment of antibodies and various ligands has also been demonstrated.^{30,79} Drug loaded liposomes with encapsulated or surface-functionalized gadolinium or fluorophores have been used to simultaneously image tumors during nanoparticle-targeted drug delivery.^{80–82}

Biodegradable polymer nanocarriers have also been investigated as a means of encapsulating drugs and releasing them over time. Both poly dl-lactide co-glycolide (PLGA) and polylactide (PLA) nanoparticles have been formed that immobilize drugs dispersed in their matrix and release them upon degradation.^{24,83} Other polymers, including polyethyleneimine (PEI), polylysine, and cyclodextrin-containing polymers, are used to condense DNA or RNA into nanoparticle carriers that can be targeted to cancer cells for gene or siRNA delivery.³⁵ Polymeric micelles consist of amphiphilic block copolymers that self-assemble into a water-soluble nanoparticle with a hydrophobic core. These can be used to encapsulate water-insoluble drugs such as doxorubicin and adriamycin and targeted to tumors.^{84–86} Polymersomes are another variation of polymer-based nanoparticulate vesicles that self-assemble from amphiphilic block copolymers.⁸⁷ These have been used to encapsulate doxorubicin with well-controlled release over several days.^{88,89}

Another class of nanoparticle-based drug carriers are dendrimers. These consist of a network of branching chemical bonds around an inner core. One of the more popular dendrimers, polyamidoamine dendrimers (PAMAMs), are nonimmunogenic, water-soluble, and possess terminal amine functional groups for conjugation of a variety of surface moieties.⁹⁰ Their inner core can be used to encapsulate anti-cancer drugs such as doxorubicin and methotrexate.⁹¹ Drugs may also be conjugated to the dendrimer surface along with ligands for targeting.^{92,93} A dendrimer functionalized with FITC, folic acid, and methotrexate has been synthesized to have imaging, targeting, and drug delivery capabilities (Figure 5.6).^{4,7,94} The synthesis of these conjugates in a scalable and reproducible manner has been described for potential clinical applications.⁴

Other nanoparticulate carriers, including nanoemulsions, drug nanocrystals, and polyelectrolyte carriers, have been developed. Nanoemulsions are formed by dissolving a drug in a lipid, cooling the solution under high pressure, and using homogenization to form solid nanoparticle lipid carriers at body temperature. Homogenization techniques can also be used to form crystalline nanosuspensions of drugs.⁷⁴ These formulations increase drug solubility and control release kinetics of the drug in the blood and at the tumor site. Polyelectrolyte carriers formed by the

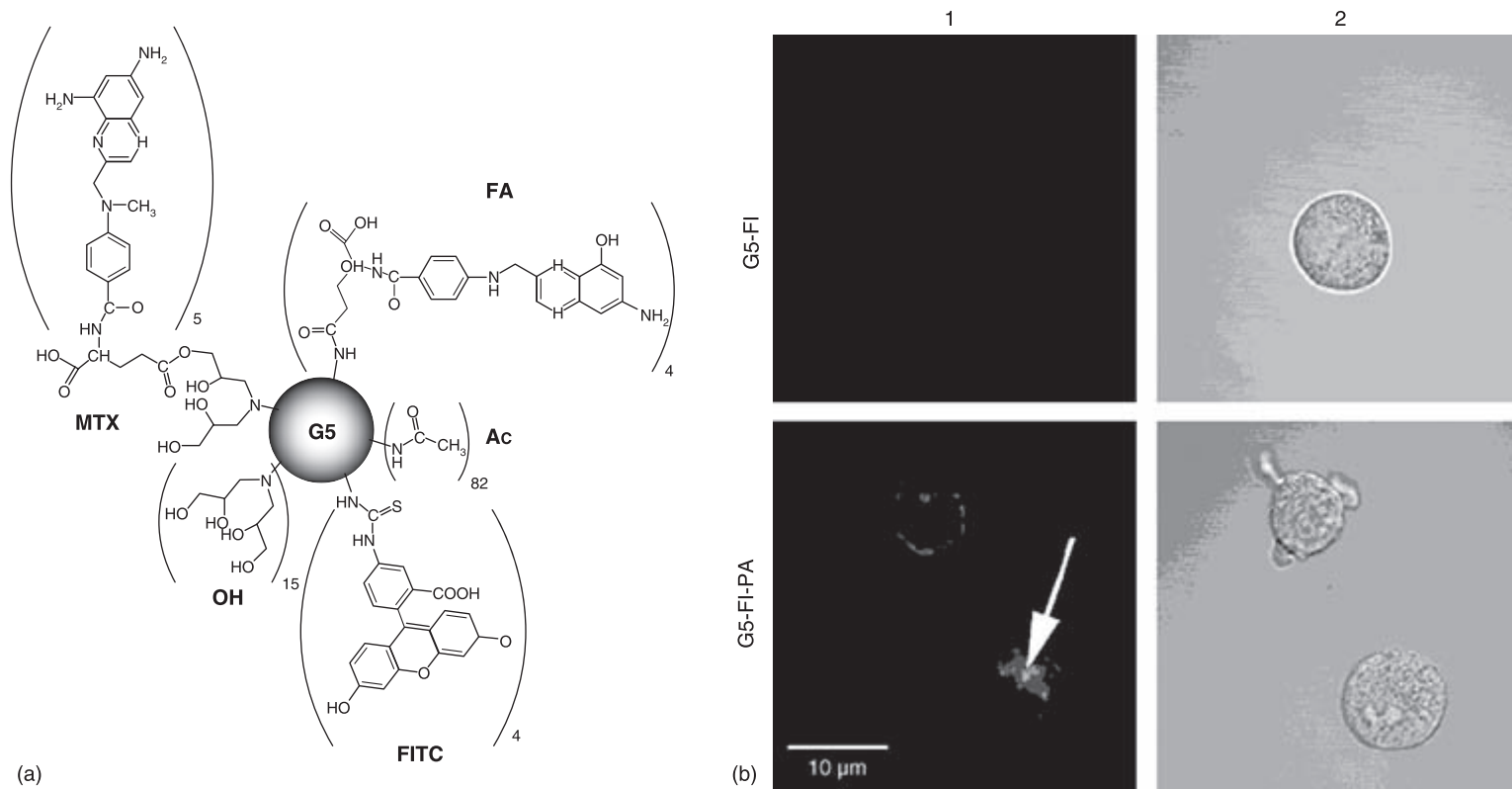


FIGURE 5.6 Multifunctional dendrimers for targeting, imaging, and drug delivery. (a) Schematic of a multifunctional dendrimer labeled with fluorescein (FITC) for imaging, folic acid (FA) for targeting, methotrexate (MTX) for therapeutic delivery, and alcohol (OH) and acetylated (Ac) moieties for particle stabilization. (b) Confocal images of FITC-labeled dendrimers incubated over cells with and without a targeting antibody, anti-PSMA (PA). (From Majoros, I. J., Thomas, T. P., Mehta, C. B., and Baker, J. R., *Journal of Medicinal Chemistry*, 48, 5892–5899, 2005; Thomas, T. P. et al., *Biomacromolecules*, 5, 2269–2274, 2004.)

layer-by-layer absorption of polycationic and polyanionic moieties can be used to encapsulate therapeutic cargo, particularly larger agents such as peptides and oligonucleotides.⁹⁵

A clever combination of drug-release modalities was recently demonstrated by the creation of a dual drug-release nanoparticle having a PLGA polymer core encapsulating doxorubicin and a PEG-lipid block copolymer shell loaded with the combretastatin (Figure 5.7).⁵ The lipophilic anti-angiogenesis drug, combretastatin, intercalates in the nanoparticle membrane and releases rapidly upon association with tumor endothelial cells, while the slower-releasing doxorubicin increases cytotoxic killing of tumor cells for a prolonged time after the vasculature shuts down. This novel system demonstrates the feasibility of integrating multiple functionalities of drug delivery on a single nanoparticle to enhance therapeutic efficacy.

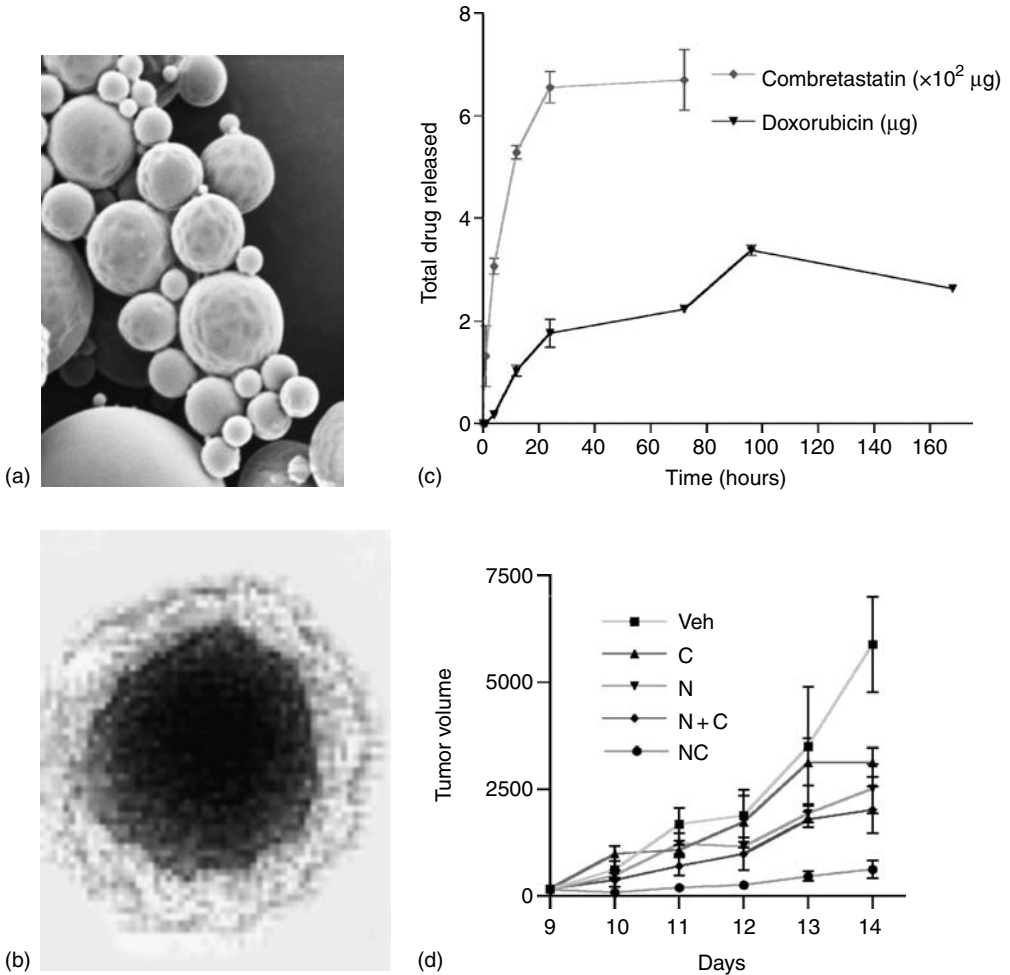


FIGURE 5.7 Dual drug-release nanoparticle for combined anti-angiogenesis and anti-cancer treatment. (a) Scanning electron micrograph showing nanocores prepared from doxorubicin-coupled PLGA. The nanocores are encapsulated inside a lipid coat, which is also loaded with an anti-angiogenesis agent. (b) Cross section of a nanocell with the dark nanocore. The lipid coat is surface-modified through pegylation, which confers stealth characteristics to the nanocell from the RES. (c) The composition of the nanocell enables a spatiotemporal release of the two agents in an acidic pH mimicking the tumor environment, as shown in the graph. (d) In vivo studies using F10 melanoma clearly show that the spatiotemporal release from the nanocells (NC) achieves better outcome than the doxorubicin-loaded nanocore or the lipid-entrapped combretastatin (C) alone, or combinations of both (N+C).⁵

5.2.5 REMOTE ACTUATION

Temporal and spatial control of therapeutic administration is important for eliminating off-target toxicity and achieving optimal delivery. Temporally controlled release profiles may be designed into nanoparticle carriers mentioned previously and spatial control can be improved with targeting. However, off-target effects, including eventual accumulation of nanoparticles in RES organs, limit many aspects of these methods of control. The ability to trigger the therapeutic activity of administered nanoparticles remotely could be a valuable tool for localizing treatments to a diseased site. Many inorganic nanocrystals and nanoemulsions used for imaging contrast absorb electromagnetic or ultrasonic energy that can also be used to remotely heat or trigger drug delivery.

Thermal ablation of tumors by nanoparticles that absorb external energy has been demonstrated both with iron-oxide nanoparticles and gold nanoshells. Superparamagnetic iron-oxide nanoparticles under the influence of an alternating electromagnetic (EM) field heat by *Brownian relaxation*, where heat is generated by the rotation of particles in the field, and *Neel relaxation*, where the magnetic domains are moved away from their easy axis with the resultant energy being deposited as heat in the solution.^{96,97} Nanoparticle concentrations of 0.1–1% are required to achieve critical temperatures for tumor ablation.^{98,99} Ongoing work to increase the absorption of magnetic nanoparticles using clinically safe RF frequencies and to increase the concentration of particles that can be targeted to the tumor may extend the utility of this technique. Alternatively, near-infrared-absorbing gold nanoshells targeted to the tumor can be used to thermally ablate the cancer cells upon illumination with a high intensity laser.^{100,101} This technique can be applied to solid tumors in close proximity to the skin, but cannot be applied to deeper lesions because of tissue absorbance.^{53,100,101} By synthesizing nanoshells with a plasmon resonance that has both absorption *and* scattering profiles, these nanoparticles may be capable of both heating and imaging tumors.⁵³

Remotely-triggered release of a therapy by heating is a promising extension of the use of nanoparticles that can absorb external energy. An example of this has been demonstrated with a model drug linked to an iron-oxide nanoparticle via a heat-labile tether that is released and diffuses into the peripheral tissue after irradiation with RF energy.⁹⁸ By modifying the susceptibility of the linker, it is possible to tune the release profile over a range of temperatures and to enable repeated administrations. The iron core of these drug-releasing nanoparticles can be used simultaneously for imaging with MRI. Additionally, the magnetic properties of these nanoparticles can be manipulated by magnetic field gradients to target sites near externally- or internally-placed magnets.¹⁰²

Drug activation using EM energy has been explored extensively with photodynamic therapy (PDT). PDT agents, when irradiated by light, produce reactive oxygen species that are toxic to cells. Agents such as porphyrins have been conjugated to various nanoparticle cores including dendrimers, liposomes, and polymers.^{103,104} When excited by light, these nanoparticles can produce enough reactive oxygen species to kill tumor cells.⁶⁰ The inherent fluorescent properties of many PDT agents enable simultaneous imaging with therapeutic delivery. A multifunctional nanoparticle platform combining MRI contrast and photodynamic therapy has been used to target, image, and treat brain cancer in a rat model.¹⁰⁵ In the future, integrating these nanoparticles with peptides capable of targeting tumors *and* subcellularly localizing them to the nuclei or mitochondria of tumor cells may enhance the therapeutic efficacy of these treatments.

Other forms of externally applied energy such as ultrasound and x-ray radiation provide alternative mechanisms to achieve remote actuation. Acoustic energy has been shown to enhance the delivery of lipid drugs from a perfluorocarbon emulsion targeted to cell membranes and from doxorubicin-loaded polymeric micelles.^{106,107} Atomically dense nanoparticles have been shown to increase the absorption of x-ray radiation, enhancing their destructive effect in surrounding tissue.¹⁰⁸ There is potential for simultaneous imaging and therapeutic delivery with these particles also.

5.3 CHALLENGES IN INTEGRATING MULTIPLE FUNCTIONALITIES AND FUTURE DIRECTIONS

Although remotely actuated nanoparticle cores such as iron-oxide and metal nanoshells naturally lend themselves to dual-imaging and therapeutic applications, the combination of imaging and other functionalities using other nanoparticle cores can be challenging. There are inherent trade-offs when combining many functional groups into one nanoparticle. In many cases, a limited number of attachment sites are available on the particle surface, making it difficult to couple several functional groups in sufficient concentration for each to function. Moreover, some groups may interact to sterically shield or alter the activity of one another when combined in close proximity. Multiple functional moieties on a nanoparticle may also reduce colloidal stability or adversely affect its *in vivo* pharmacokinetics. With significant characterization and fine tuning, dendrimers that combine targeting, imaging, and therapeutic moieties on their surface have been synthesized successfully.⁴ Similar efforts will be necessary to achieve other multifunctional nanoparticles with decorated-surface moieties.

An alternative strategy to consolidate multiple functionalities onto a single particle is to use core-shell architecture. In this case, an outer shell with one functionality, such as targeting, may be unveiled to reveal an inner core that performs a secondary function such as endosomal escape or drug release. This has been demonstrated with the conjugation of targeting moieties or protective PEG groups on the surface of dendrimers or polymers via acid-labile chemistries that degrade in the lower pH of the endosome and unveil endosomal escape mechanisms on the particle core.^{109,110} This has also been demonstrated with protease-cleavable linkers that release protective polymers on the surface of complementary nanoparticles to initiate their self-assembly.⁷³

The synthesis of nanoparticles with polar domains is another strategy that could be used to incorporate multiple functionalities on a single particle. Janus nanoparticles—named for Janus, the Roman God of doorways typically depicted with faces on the front and back of his head—have been engineered with two chemically distinct hemispheres or surfaces. These nanoparticles may be spherical (with opposing faces of unique composition), dumbbell-shaped (with two equal-sized spheres linked together), snowman-shaped, and may have other morphologies as well.^{95,111} The creation of nanoparticles with spatially separated chemical domains is a step towards replicating the controlled polarity exhibited in nature across many length scales. Separate hemispheres may be used to isolate and organize functional domains on nanoparticles such that they may simultaneously carry targeting molecules, endosomal escape domains, sensing moieties, hydrophilic and hydrophobic therapeutics, or contrast agents that otherwise might be mutually inhibitory if randomly incorporated. Moreover, there may be specific applications for which the polarity and anisotropy of Janus nanoparticles have benefit, such as real-time detection of oriented binding events, targeted bridging of multiple components at a tumor cell, directed drug delivery, or guided self-assembly.

Although there have been many exciting advances in the application of nanoparticles for cancer imaging and treatment, the true power of these materials will be in their ability to interact with disease processes intelligently. The modular design of functionalities that target, sense, signal, and treat and the ongoing efforts to consolidate these into single nanoparticle platforms is one way in which such ‘smart’ materials are being developed. The further elucidation of complex biological processes in tumorigenesis, the discovery of nanomaterials with other novel properties, and the consolidation of biological and synthetic machinery in these materials in new and elegant ways are key factors that will determine their future success in cancer therapy.

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