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Review

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Current Advances in Polymer-Based Nanotheranostics for Cancer Treatment and Diagnosis

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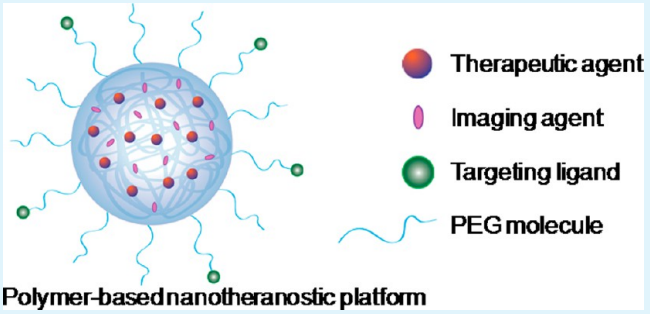
ABSTRACT: Nanotheranostics is a relatively new, fast-growing field that combines the advantages of treatment and diagnosis via a single nanoscale carrier. The ability to bundle both therapeutic and diagnostic capabilities into one package offers exciting prospects for the development of novel nanomedicine. Nanotheranostics can deliver treatment while simultaneously monitoring therapy response in real-time, thereby decreasing the potential of over- or under-dosing patients. Polymer-based nanomaterials, in particular, have been used extensively as carriers for both therapeutic and bioimaging agents and thus hold great promise for the construction of multifunctional theranostic formulations. Herein, we review recent advances in polymer-based systems for nanotheranostics, with a particular focus on their applications in cancer research. We summarize the use of polymer nanomaterials for drug delivery, gene delivery, and photodynamic therapy, combined with imaging agents for magnetic resonance imaging, radionuclide imaging, and fluorescence imaging.



KEYWORDS: *polymer, nanoparticle, cancer, theranostics, drug delivery, imaging*

1. INTRODUCTION

Originally introduced by Funkhouser in 2002, the term "theranostics" describes any "material that combines the modalities of therapy and diagnostic imaging" into a single package.[1] More recently, theranostics has become commonly used to describe image-guided therapy, or therapeutic agents that concomitantly possess imaging capabilities. The traditional approach to treating patients involves first diagnosing a patient and then subsequently utilizing a certain known therapy to treat the disease or disorder. As a result, medical research has focused heavily on characterizing a type of disease and then developing a drug or standard treatment regimen for that general disease.[2] While this approach is still typically the clinical standard, many of the most vexing diseases, such as cancer, are heterogeneous in their expression,[3,4] necessitating more individualized and tailored methods of treatment.[5] Toward this end, the field of nanotheranostics has emerged, which aims to allow physicians to simultaneously monitor drug distribution and release and evaluate therapeutic efficacy noninvasively and in real-time. This valuable information would enable physicians to better tailor treatment plans based on each patient's individual responses and needs, thereby lowering the chances of the patient experiencing adverse side effects due to over- or under-dosing.[6]

A number of nanosized delivery vehicles have been studied for theranostic applications. For example, chemotherapeutic agents have been conjugated onto gold[7,8] and iron oxide nanoparticles.[9,10] Quantum dots (QDs) with their inherent fluorescence have also been utilized for image-guided therapies.[11−13] Carbon nanotubes, another widely studied inorganic material, have also been presented as a potential candidate for concurrent optical imaging and drug/gene delivery.[14,15] While these platforms have shown promising results in animal models, their inorganic or metallic nature has raised concerns of toxicity, immunogenicity, and slow excretion kinetics from the body, which need to be thoroughly examined prior to clinical test in human. Silver-based nanoparticles, for example, can cause adenosine 5′-triphosphate depletion and mitochondrial damage,[16] while single-walled carbon nanotubes can cause oxidative stress and trigger apoptosis;[17] magnetic nanoparticles can induce cell death through membrane damage.[18] In this review, we will focus on the theranostic potential of polymer-based nanomaterials, which possess excellent biocompatibility, biodegradability, and structural versatility.[19,20] Biopolymers naturally degrade into safe materials (i.e., carbon dioxide and water) over time in the body, and are typically nontoxic except at extremely high concentrations. Polymers such as polyethylene glycol (PEG), poly(D,L-lactic acid), poly(D,L-glycolic acid), and poly(ε-caprolactone) have already been approved for clinical use in macroformulations. Polymer-based platforms have been studied extensively for cancer therapy and offer many advantages.[21] In particular, polymeric nanoparticles are able to enhance drug

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Table 1. Overview of Commonly Used Imaging Modalities

imaging modality	imaging agent	spatial resolution	advantages	disadvantages
MRI	gadolinium, iron oxide, manganese oxide, ^{19}F-labeled compounds	10−100 μm	clinical translation; high resolution; no radiation; no depth limit; quantitative results	high cost; long imaging time; limited to patients with no metal implants or tattoos
PET	^{18}F, ^{64}Cu, ^{11}C, ^{15}O-labeled compounds	1−2 mm	clinical translation; high sensitivity; unlimited penetration; image biochemical processes; quantitative results	high cost; radiation; low resolution
SPECT	99 mTc, ^{111}In chelates	1−2 mm	clinical translation; high sensitivity; unlimited penetration; relatively lower cost; quantitative results	radiation; low resolution
optical imaging	fluorochrome, photoproteins	1−5 mm	high sensitivity; no radiation; high-throughput screening for target confirmation and compound optimization; multichannel imaging	limited clinical translation; low resolution; low depth penetration
ultrasound	nano/microbubbles	50 μm	high resolution; low cost; ease of operation; no radiation; quantitative results	low depth penetration

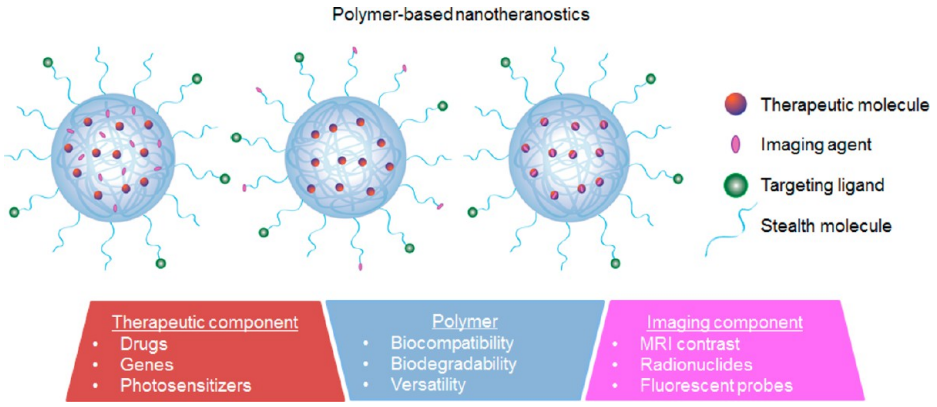

Figure 1. Schematic illustration of a polymer-based nanotheranostic platform, consisting of three major components: a biocompatible polymeric nanocarrier component, a therapeutic component, and an imaging component. Some platforms also incorporate targeting ligands for specific delivery.

efficacy compared with free drugs via improved drug encapsulation and delivery, prolonged circulation half-life, and sustained or triggered drug release.[22,23] Polymeric nanoparticles are also able to accumulate at specific disease sites through passive targeting by the enhanced permeability and retention (EPR) effect, or through active targeting by the incorporation of targeting moieties specific for a receptor or cell surface ligand at the region of interest.[24−26]

Building on the progress made in developing therapeutic polymeric nanoparticles, researchers are now incorporating clinically used imaging modalities into therapeutic nanocarriers.[27] Among these are magnetic resonance imaging (MRI) contrast agents, radioactive agents for radionuclide imaging via positron emission tomography (PET) or single photon emission computed tomography (SPECT), fluorescent agents for fluorescent imaging, and nano/microbubbles for ultrasound imaging. Each modality has its own advantages and disadvantages (Table 1), which need to be weighed to determine the most appropriate imaging technique for the desired outcome. MRI offers high spatial resolution and soft tissue contrast without tissue-penetrating limitations and is widely used in hospitals, but is expensive and time-consuming. Radionuclide imaging is also commonly used in the clinic and offers high sensitivity with unlimited tissue penetration. On the other hand, it is also high cost and offers limited spatial resolution compared with MRI. Fluorescence imaging provides a means for high-throughput screening for target confirmation and compound optimization along with high sensitivity and multicolor imaging, but has low tissue penetration and spatial resolution.[28] Ultrasound imaging offers high resolution at much lower cost than MRI or PET/SPECT, but has low depth penetration.

In general, a polymer-based theranostic material is comprised of at least three main components: (i) a polymer component that offers stabilization and biocompatibility, (ii) a therapeutic agent (i.e., small-molecule drug, siRNA, etc.), and (iii) an imaging agent (i.e., MRI contrast agent, radionuclide, fluorophore, etc.) (Figure 1). In some cases, the therapeutic component can also act as the imaging component, such as doxorubicin, which possesses an inherent fluorescence.[29] These components can be arranged in different ways depending on the specific delivery platform. Many formulations now also incorporate targeting ligands as a fourth component to further enhance specific delivery to the tumor site. In this article, we will provide a review on current advances in using polymeric nanoparticles for drug delivery, gene delivery, and photodynamic therapy, combined with imaging agents for magnetic resonance imaging, radionuclide imaging, and fluorescence imaging.

2. COMBINED DRUG DELIVERY AND IMAGING

Prior to the development of nanomedicine, traditional drug delivery has faced many obstacles, primarily related to poor pharmacokinetics and undesirable biodistribution of drug. In particular, typical chemotherapy drugs suffer from low solubility, *in vivo* degradation, accelerated *in vivo* clearance rate, and inability to cross biological barriers.[30,31] Polymer-based nanostructures provide an attractive solution to overcome these problems by enabling the ability to precisely control the location, dose, and time of delivering therapeutics.

Table 2. Polymer-Based Theranostic Systems Containing SPIO for Anticancer Drug Delivery

polymer	drug	reference
pluronic-based polymers	curcumin, DOX, paclitaxel	134−136
PEG−PMAA−poly(glycerol monomethacrylate) block copolymers	ADR	137,138
PEG−PGA−PEG−acrylate	DOX	139
PEG−PLA−PEG−acrylate	DOX	140
PVA	5-FU, DOX, ceftriaxone	141−143
poly(NIPAAm)	DOX	144
PLA, PLGA	IFNα-2b	145
PEO-*b*-PLGA-*b*-PEO	DOX	146
PEG−PCL	DOX	147

A variety of polymeric nanoparticles—including polymer conjugate complexes,[32,33] nanospheres,[34−36] micelles,[37−39] and dendrimers[40−42]—have been developed to aid in the delivery of drugs to cancerous sites and have shown great efficacy against various types of cancers. Conjugation of drug molecules to the polymer backbone allows for precise drug loading and control over release kinetics.[43] Self-assembled nanospheres, micelles, and dendrimers loaded with therapeutic agents offer sustained and controlled release through surface or bulk erosion, drug diffusion through the polymer matrix, or environmental activation or stimulation.[44] By combining an imaging agent along with the encapsulated drug within a polymeric nanoparticle, researchers have been able to achieve analysis of drug distribution and release at the target site in real time. The live evaluation of drug distribution provides assistance in predicting drug response and can better facilitate treatment regimens to be specifically tailored for each individual.

2.1. Drug Delivery and MRI. MRI is a commonly used radiology technique to analyze tissues, and it offers high spatial resolution without the danger of ionizing radiation. In MRI, a magnetic field is applied at an appropriate resonant frequency that excites hydrogen atoms in the tissues. The excited hydrogen atoms give off a radiofrequency signal as they return to their equilibrium state, which is detected and transformed into an image. Each tissue's hydrogen atoms relax at different rates, which provide contrast between different tissues. A variety of paramagnetic and superparamagnetic metals are commonly used as contrast agents for MRI. Among these, superparamagnetic iron oxide (SPIO) and gadolinium (Gd) are most often utilized. SPIO and Gd interact with an external magnetic field to improve the visibility of internal structures by altering the relaxation times of atoms in tissues where they are present. Polymeric nanoparticles have been shown to be effective carriers of both SPIO and Gd.[45−47]

Superparamagnetic iron oxide is used as a T2 (spin−spin) contrast enhancement MRI agent.[48] To create T2-weighted images, the magnetization is allowed to decay for different amounts before measuring the MR signal by changing the echo time. T2-weighted images are often used to study pathology as fluid appears bright against the darker normal tissue. Various polymer-based SPIO-containing drug delivery systems have been developed in the form of nanospheres, micelles, nanogels, and polymersomes (Table 2). These systems offer narrow particle size distribution, biocompatibility, good stability, prolonged blood circulation times, high drug loading, and control over drug release rate, in addition to superparamagnetic behavior for MRI contrast. For example, SPIO and chemotherapy drug doxorubicin (DOX) can both be directly encapsulated by using an amphiphilic block copolymer composed of maleimide−PEG−poly(lactic acid). This block copolymer self-assembles into nanoparticles with functionalizable maleimide groups on the surface, which allows for further conjugation of targeting peptides.[49] In tumor-bearing mice, these targeted theranostic nanoparticles showed increased tumor-specific accumulation and enhanced inhibition of tumor growth. In another example, biodegradable poly(lactic-*co*-glycolic acid) (PLGA) was used to encapsulate docetaxel and SPIO by Ling et al. (Figure 2).[50] Drug-release experiments

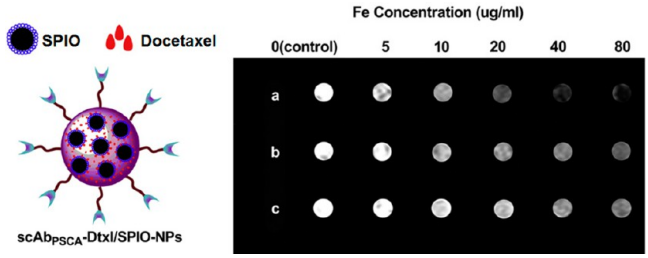

Figure 2. Targeted theranostic PLGA nanoparticles dual-loaded with SPIO and docetaxel for concurrent MR imaging and cancer therapy. (left) A schematic of the nanotheranostic formulation. (right) T2-weighted imaging of PC3 cells (1×10^6) after 2 h of incubation with (a) targeted SPIO/docetaxel-loaded nanoparticles, (b) nontargeted SPIO/docetaxel-loaded nanoparticles, and (c) Endorem (a commercially available MRI contrast agent) at Fe concentrations of 0, 5, 10, 20, 40, and 80 μg/mL; cells were then mixed with 2% agarose solution in PBS and scanned under a 1.5 T MRI scanner at room temperature. Reproduced with permission from reference 50.

showed sustained release with no initial burst effect, and PC3 prostate cancer cells treated with targeted docetaxel- and SPIO-loaded PLGA nanoparticles showed high intracellular iron concentration with strong contrast in T2-weighted MRI. Yang and colleagues demonstrated the ability to target breast cancer *in vivo* using a HER2-targeted PLGA−PEG block copolymer nanoparticle encapsulating $MnFe_2O_4$ and DOX.[51] These targeted nanoparticles exhibited not only ultrasensitive detection by MRI, but also excellent tumor growth retardation both *in vitro* and *in vivo*. Other polymers such as Pluronic F-127 have also been used to create stable nanotheranostic formulations, indicating the applicability of encapsulating SPIO-drug mixtures in existing polymeric nanocarrier systems.[52]

The most commonly used T1 (spin−lattice) MRI contrast agent, gadolinium(III), in imaging systems generates positive image contrast by increasing the longitudinal relaxation rate of the surrounding water protons. In T1-weighted images, fluid appears dark, water-based tissues appear midgray, and fat-based tissues appear bright. Typically, Gd(III)-diethylenetriamine-pentaacetic acid (Gd-DTPA) or Gd-tetraazacyclododecanete-

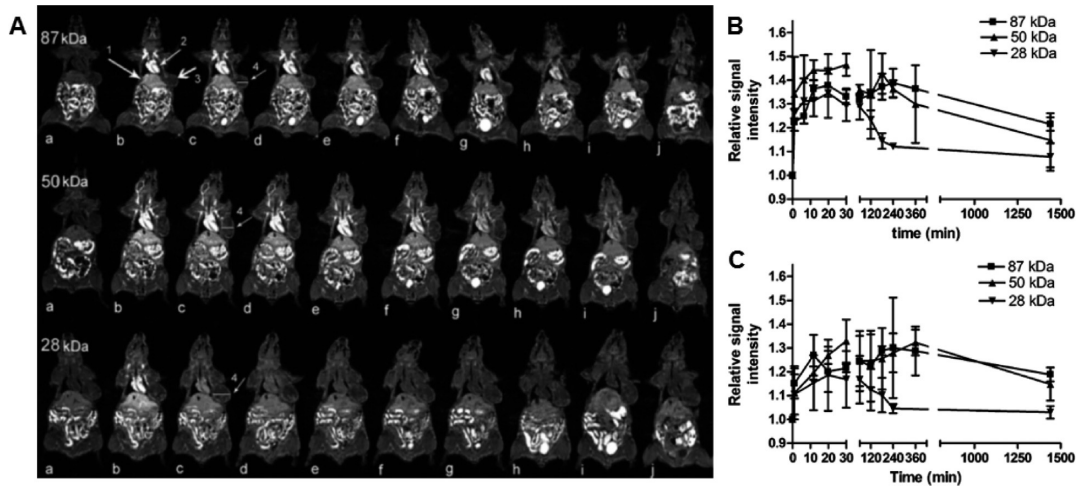

Figure 3. (A) Coronal MR images of tumor bearing mice (a) before and at (b) 1, (c) 11, (d) 20, (e) 30, (f) 60, (g) 120, (h) 180, (i) 240 min, and (j) 24 h after injection with PGA−1,6-hexanediamine−(Gd-DO3A) conjugates of different molecular weights. Higher molecular weights demonstrated increased tumor accumulation. Arrows point to the (1) liver, (2) heart, and (3) tumor tissue. (B, C) Relative signal intensity in (B) tumor periphery and (C) tumor interstitium before and at various time points after the injection of the polymer conjugates. Reproduced with permission from reference 54.

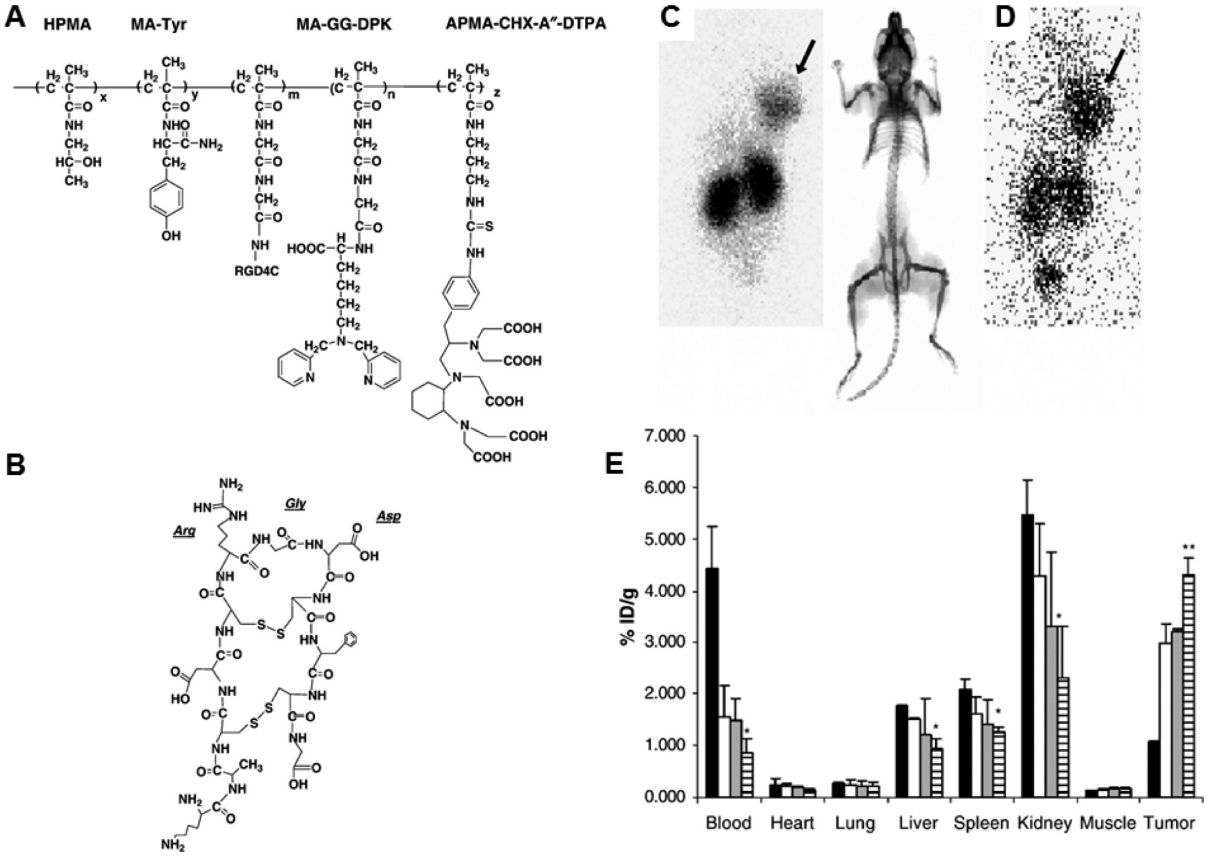

Figure 4. Structure of targeted HPMA−RGD4C conjugate and its biodistribution. (A) Chemical structure of HPMA conjugate. The side chains of MA-GG-DPK and APMA-CHX-A″-DTPA may be used to label the conjugate with ^{99m}Tc and ^{90}Y, respectively. MA-Tyr may be used to couple iodine isotopes for biodistribution or radiotherapy (^{123}I, ^{131}I). (B) Structure of RGD4C peptide. The peptide has a doubly cyclized structure via two disulfide bridges and is conjugated to the polymer backbone via the ϵ-amino group of the terminal lysine residue. (C, D) Scintigraphic images of ^{99m}Tc-labeled HPMA copolymer−RGD4C conjugate in SCID mice bearing DU145 human prostate tumor xenografts (C) 24 and (D) 48 h post intravenous injection showed marked tumor accumulation. (E) Residual radioactivity in % injected dose per gram of organ tissue 1, 24, 48, and 72 h after administration of the ^{99m}Tc-labeled HPMA copolymer−RGD4C conjugate. Reproduced with permission from reference 62.

traacetic acid (Gd-DOTA) is used in the formulation of a polymeric nanocarrier for Gd-based MRI contrast.[53] Ye et al. have conjugated Gd-DOTA to the side chains of poly(L-glutamic acid) (PGA) and demonstrated MRI signal enhancement in a breast tumor model *in vivo* (Figure 3).[54] Ye and colleagues have also examined the effect of varying molecular

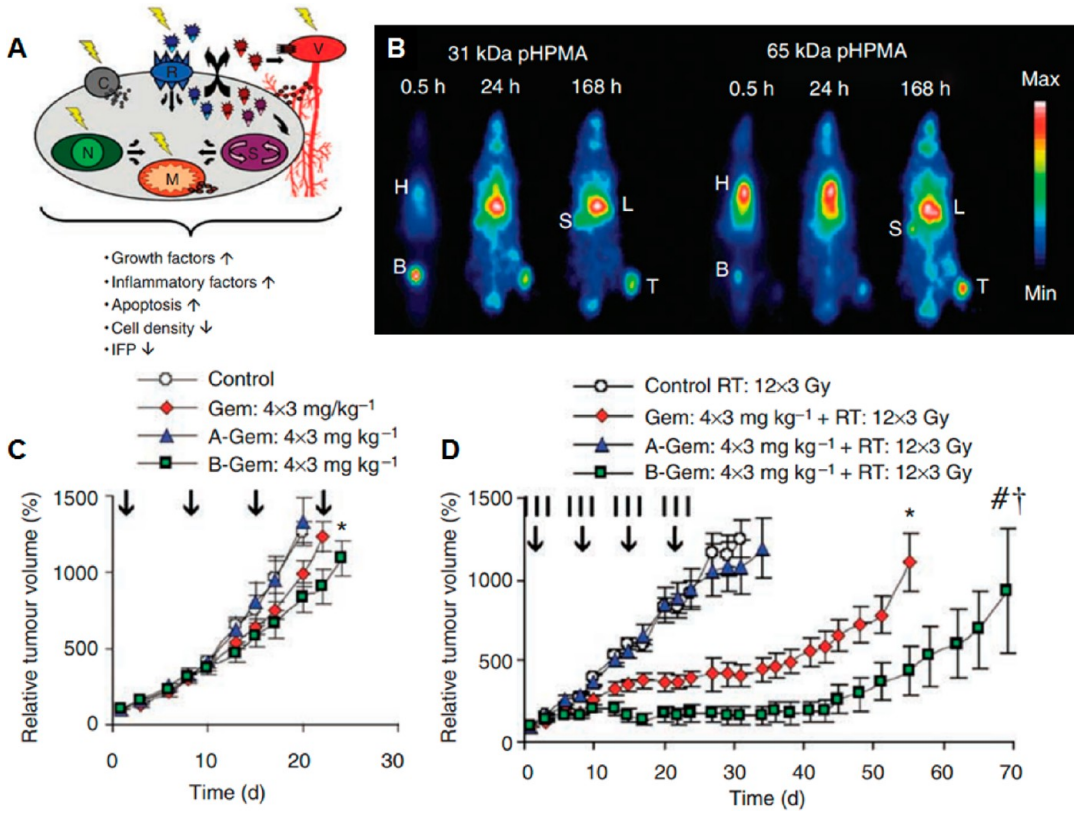

Figure 5. (A) Potential physiological mechanisms by which radiotherapy increases tumor accumulation of drug targeting systems. Radiotherapy can affect the integrity and function of the tumor vasculature (V), the expression of certain cell receptors (R), several cell membrane-related (C), nuclear (N), mitochondrial (M), and signaling (S) processes. (B) Scintigraphic analysis of the biodistribution of two differently sized iodine-131-labeled HPMA copolymers in Copenhagen rats bearing subcutaneously transplanted Dunning AT1 tumors, demonstrating prolonged circulation and effective tumor accumulation (H: heart (blood), B: bladder, S: spleen, L: liver, T: tumor). (C) Growth inhibition of Dunning AT1 tumors induced by four intravenous injections (days 1, 8, 15, and 22; vertical arrows) of saline, free gemcitabine and HPMA copolymer-bound gemcitabine. A-Gem: pHPMA-AH-Gem (20 kDa); B-Gem: pHPMA-GFLG-Gem (24 kDa). (D) Tumor growth inhibition induced by four intravenous injections of the above-mentioned chemotherapeutic agents in combination with a clinically relevant regimen of fractionated radiotherapy (12 × 3 Gy; vertical lines). Reproduced with permission from reference 63.

weights of polymer, finding that conjugates with higher molecular weights (87 kDa) exhibited more prolonged blood circulation and increased tumor accumulation over lower molecular weight conjugates (28 kDa). In another study, Liao et al. synthesized a hybrid nanoparticle system consisting of a hydrophobic PLGA core and a hydrophilic Gd-DTPA folate-coated PEGylated liposome shell for MRI and targeted drug delivery.[55] The nanoparticles showed high DOX loading efficiency and sustained release, while simultaneously offering high T1 relaxivities for high-resolution MRI via the paramagnetic Gd-DTPA chelated to the liposomal shell layer. Hong et al. have also taken a hybrid approach to nanotheranostics, combining a DOX-loaded liposomal core with an acid-sensitive cholesterol-terminated poly(acrylic acid) polymer shell functionalized with Herceptin and Gd(III).[56] Their nanoparticle formulation revealed a 120-fold increase in cellular uptake of Gd in comparison with the clinically approved and commercially available Gd-DOTA, leading to significant T1 MRI contrast enhancement. Among other polymers utilized as theranostics vehicles for simultaneous MR imaging and drug delivery in cancer applications are *N*-(2-hydroxypropyl)-methacrylamide (HPMA)-based copolymers, conjugated with Gd and therapeutic drugs,[57] and multiarm star block copolymers.[58]

2.2. Drug Delivery and Radionuclide Imaging. Radionuclide imaging is used in medicine to image the extent of disease development based on cellular metabolism and physiology within the body, rather than relying on physical changes in tissues like MRI. Similar to MRI, radionuclide imaging has high sensitivity with no tissue-penetration limitations. Radioisotopes such as ^{11}C, ^{18}F, ^{64}Cu, ^{76}Br, ^{99m}Tc, ^{111}In, and ^{90}Y are administered intravenously or orally. Gamma cameras are then used to capture and create images from the radiation emitted by the internalized radionuclides. Many such radionuclide compounds have been extensively explored along with a variety of copolymers with the goal of formulating a robust nanodelivery system.[59−61] In one example, Mitra and colleagues have conjugated ^{99m}Tc and ^{90}Y to HPMA, which was further conjugated to the $\alpha_v\beta_3$ targeting peptide RGD4C.[62] These targeted polymer−radionuclide conjugates demonstrated enhanced cell adhesion to $\alpha_v\beta_3$ expressing endothelial cells as well as antitumor efficacy against a SCID mouse xenograft model of human prostate carcinoma (Figure 4). Lammers et al. have also taken advantage of HPMA to load ^{131}I, along with antitumor agent doxorubicin or gemcitabine, to study the dual imaging and therapeutic capabilities of drug- and radionuclide-loaded polymeric nanocarriers.[63] These polymeric drug carriers demonstrated prolonged circulation time and selective accumulation in the tumor site. The two components acted

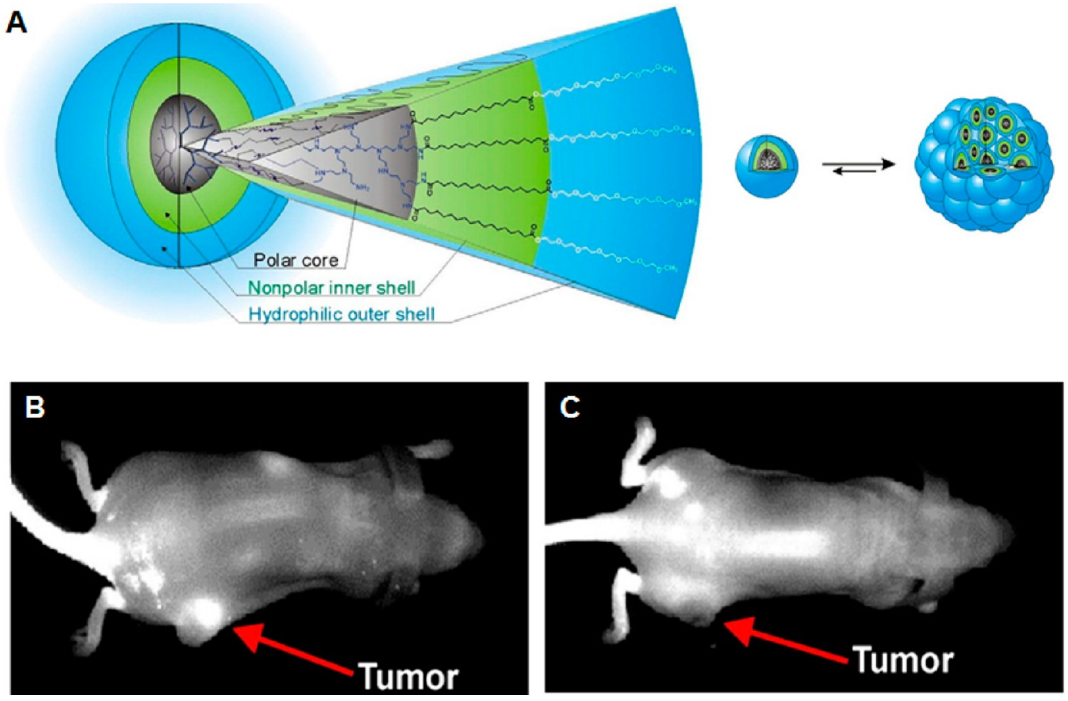

Figure 6. (A) Dendritic core−multishell architecture with PEI core, inner hydrophobic segment, and terminal mPEG chain. Dynamics between the nanoparticle unimers and their aggregates are responsible for encapsulation of therapeutic drugs. (B) Strong contrast was observed 6 h after administration of ITCC dye-loaded nanoparticles to F9 teratocarcinoma-bearing mice. (C) Much less contrast was observed using free dye. Reproduced with permission from reference 71.

synergistically to increase the therapeutic efficacy against the tumor (Figure 5). As such, combining both chemotherapy and radiotherapy into a single nanocarrier can be an effective method to combat solid tumors.

2.3. Drug Delivery and Fluorescence Imaging. Fluorescence spectroscopy has been a powerful tool utilized throughout the years in many different fields, such as biochemistry, molecular biology, engineering, and nanomedicine. Many biomolecules in nature, including amino acids, proteins, and lipids, possess the inherent ability to fluoresce when excited with UV−vis light.[64] The photons emitted from naturally fluorescing biomolecules or from externally administered fluorescent probes can be harnessed for imaging. Fluorescence imaging offers a low-cost technique with good spatial resolution in the UV−near-infrared (NIR) wavelength range and is on par with the sensitivity of radioisotopes used in PET and SPECT.[65,66] Its weaknesses include limited tissue penetration, potentially high noise and background from tissue scattering of photons in the visible region, tissue autofluorescence, light absorption by proteins, and interference from water molecules.[67] However, the use of NIR light for *in vivo* imaging overcomes some of these challenges, offering tissue penetration up to several centimeters and reduced autofluorescence and tissue scattering.[68,69]

NIR fluorescent probes have been incorporated into hyperbranched polyhydroxyl polymeric nanoparticles along with apoptosis-initiating protein cytochrome *c* by Santra et al.[70] These nanoparticles were targeted using folic acid ligand, and they demonstrated enhanced uptake and therapeutic effect against various human carcinoma cells *in vitro* while also emitting photons for imaging via excitation of encapsulated indocyanine green (ICG). Also using a indocyanine-based dye along with Nile red, Quadir and co-workers have prepared dendritic core−multishell nanoparticle composed of hyperbranched polyethylenimine (PEI) conjugated to monomethyl PEG to encapsulate and transport three different antitumor drugs (DOX, methotrexate, and sodium ibandronate) (Figure 6).[71] When injected into F9 teratocarcinoma bearing mice, the core−multishell nanoparticles demonstrated a strong contrast within the tumor tissues compared to free dye 6 h after administration. Hu et al. have successfully synthesized a multifunctional micelle with fluorescent imaging and drug delivery capabilities.[72] Multifunctional micelles were prepared via the coassembly of DOX-conjugated monomethoxyl PEG-*block*-poly(L-lactide-*co*-mercaptoethanol) copolymer, rhodamine B-conjugated mPEG-*b*-p(LA-*co*-ME), and folic acid-conjugated PEG-*b*-PLA copolymer. *In vivo* fluorescence imaging experiments in mice with hepatocarcinomas demonstrated that the folic acid-conjugated micelles accumulated for longer periods in tumor tissues and exhibited enhanced antitumor efficacy compared with either free DOX or a nontargeted micelle formulation. Many researchers also take advantage of the inherent fluorescence of doxorubicin for therapeutic studies and have combined multiple imaging modalities together in conjunction with drug therapy.[73,74]

QDs have also been used as a potential fluorescent agent for theranostic applications.[75,76] QDs are luminescent semiconductor nanocrystals that are typically composed of periodic groups II−VI (i.e., CdSe and CdTe) or III−V (i.e., InP and InAs) semiconductor materials. QDs have a narrow, symmetric, and size-tunable emission spectra and broad excitation spectra, making them particularly valuable for multicolor fluorescent applications. The benefits of using QDs are that they typically exhibit much stronger fluorescence as well as higher fluorescence stability against photobleaching compared with organic fluorophores or fluorescent proteins.[77,78] This robust stability allows QDs to be used for prolonged fluorescence monitoring in living organisms. Song et al. have reported the

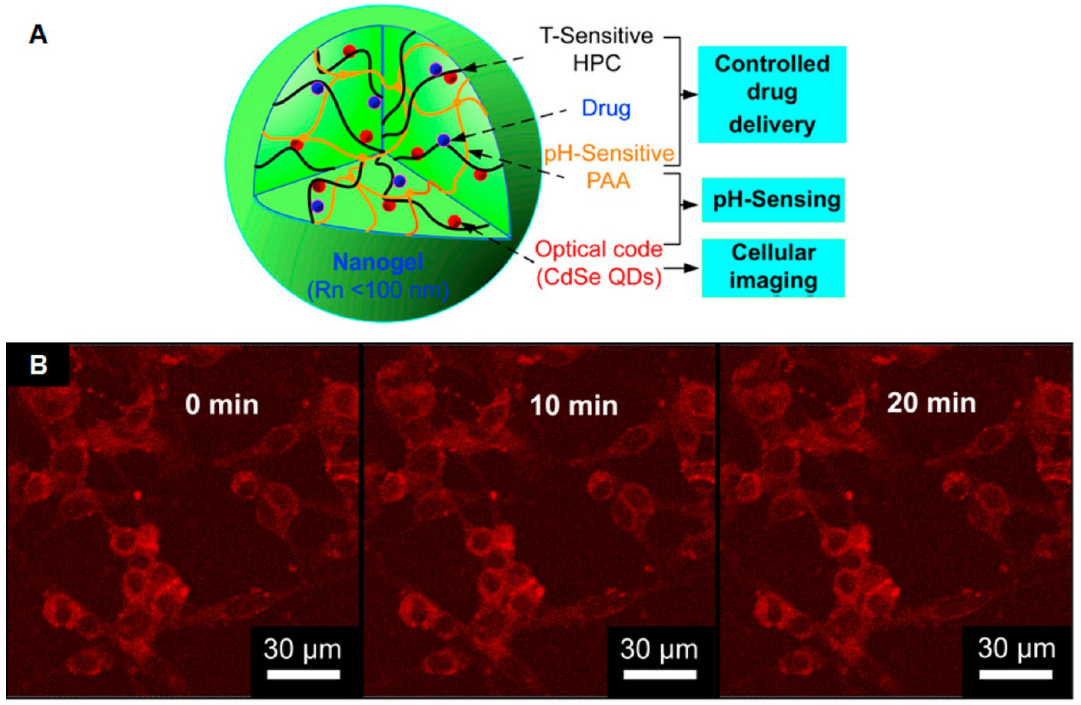

Figure 7. Schematic and fluorescence microscopy images of QD-loaded nanogels. (A) Design of hydroxypropylcellulose-poly(acrylic acid) hybrid nanogels loaded with CdSe QDs for pH and temperature dual-responsive multifunctional applications in biomedicine. (B) Scanning confocal fluorescence images of mouse melanoma B16−F10 cells after incubation with QD-loaded nanogel. Reproduced with permission from reference 80.

fabrication of HIF-1α (hypoxia inducible factor-1α) antibody-conjugated pluronic triblock copolymer micelles loaded with paclitaxel and CdTe QDs.[79] This targeted micelle formulation selectively killed HIF-1α overexpressing stomach cancer cells, with internalization of the nanoparticles easily visualized via fluorescence microscopy. CdTe QDs have also been incorporated, along with anticancer drug temozolomide, into pH and temperature dual-responsive polysaccharide-based nanogels composed of hydroxypropylcellulose-poly(acrylic acid).[80] The pH- and temperature-triggered nanogels laden with temozolomide and QDs were effective against B16−F10 mouse melanoma *in vivo*, demonstrating sustained drug release at different pH values and prolonged intense stable photoluminescence (Figure 7). Other polymer-based theranostic systems that utilize fluorescent agents are summarized in Table 3.

2.4. Drug Delivery and Ultrasound and Photoacoustic Imaging. Ultrasound imaging and acoustic microscopy are commonly used diagnostic imaging techniques in the clinic. Ultrasound imaging makes use of sound waves at frequencies of 2 MHz or higher, with shorter wavelength allowing for the resolution of small internal details in tissues and organs. Acoustic imaging utilizes high and ultrahigh ultrasound waves with frequencies up to 4 GHz. The two modalities are used to visualize muscles, tendons, and many internal organs to investigate their size, structure, and any pathological lesions in real time. Ultrasound offers high resolution and ease of operation with relatively low costs. As a drug delivery modality, ultrasound can be targeted in precise energy deposition patterns and can be performed noninvasively or minimally invasively.

Microbubbles and nanobubbles are the most commonly used contrast agents in ultrasound for imaging inflammation, angiogenesis, intravascular thrombi, and tumors. Gao et al.

Table 3. Polymer-Based Theranostic Systems Containing Fluorescent Probes for Anticancer Drug Delivery

polymer	therapeutic component	fluorescent component	reference
poly(ethylene oxide)-*b*-poly(allyl glycidyl ether)	DOX	Dyomics 615	148
chitosan	DTX	fluorescein	149
poly(ethylene glycol)-*b*-poly(L-lysine)-*b*-poly(L-phenylalanine)	DTX	Cy5.5	150
amphiphilic star hyperbranched block copolymers	DOX	cascade blue	151
PVA	Temozolomide	Bi_2O_3 quantum dots	152
PEG-pentacosydonic acid	Herceptin	CdTe/CdSe QDs	153
chitosan	Camptothecin, DOX	CdTe, ZnO QDs	154,

have used PEG−poly(L-lactic acid) and PEG−polycaprolactone block copolymers to form micelles that encapsulated doxorubicin. Perfluoropentane (PFP) was added, and the solution was sonicated, resulting in a mixture of doxorubicin-loaded micelles and doxorubicin-loaded, PFP-encapsulating nanobubbles.[81] This mixture was shown to accumulate in an *in vivo* model of breast cancer. Upon accumulation, the mixture formed microbubbles, which cavitated and collapsed upon tumor-directed ultrasound. This led to localized drug release and tumor regression while simultaneously enabling molecular imaging of the nanobubbles.

Photoacoustic imaging technology has recently emerged as a novel method that allows for the visualization of molecular imaging probes with high performance. Upon absorption of light pulses of ultrashort duration, photoacoustic contrast

agents in the tissue allow the absorbed energy to undergo thermoelastic expansion that emits mechanical waves at ultrasonic frequencies. These mechanical waves can then be detected by ultrasonic transducers to form images. Pu and colleagues have recently reported the use of NIR light-absorbing semiconducting polymer nanoparticles as a new class of contrast agents for photoacoustic molecular imaging.[82] The nanoparticles produced a stronger signal than the commonly used gold nanorods and single-walled carbon nanotubes, providing real-time *in vivo* imaging of reactive oxygen species. Combined with a therapeutic agent, this platform would be a powerful theranostic tool.

3. COMBINED GENE THERAPY AND IMAGING

Gene therapy is the transfer of specific genetic material to target cells in a patient for the ultimate purpose of preventing or altering a particular disease state. Studied extensively in the past few decades, gene therapy has the potential to treat a variety of acquired and inherited genetic disorders such as diabetes, blindness, Parkinson's disease, and cancer.[83] The goal of gene therapy is to replace, repair, regulate, or silence a defective gene through the administration of defined genetic material (i.e., DNA or siRNA). Currently, however, there are a number of technical challenges underlying gene therapy. First, gene therapy as of now is short-lived. Genetic material introduced into the target cell can be enzymatically degraded, preventing long-term benefits and necessitating multiple administrations of gene therapy. Second, the existing delivery mechanisms (typically viral vectors) oftentimes induce an immune response in patients, making it difficult for gene therapy to be repeated in patients. Third, there is a possibility of inducing a tumor if administered DNA is integrated into the wrong location in the genome, though the recent development of clustered regularly interspaced short palindromic repeats (CRISPR) technology significantly decreases this possibility.[84,85] By inserting a plasmid containing cas genes and precisely designed CRISPRs (which are evolutionarily conserved), an organism's genome can be edited via addition or deletion of DNA base pairs at any desired location. Fourth, the longer the therapeutic DNA strand is, the more difficult it is to efficiently incorporate it into cell genomes. Lastly, the current cost of gene therapy is extremely high. To address these concerns, researchers have begun to use polymer-based systems for the delivery of genetic material because polymers are more cost-effective, safer, and relatively easy to tailor.[86] Generally, there are two types of polymer systems currently in use for gene delivery. In the first, the polymer carries the genetic material (i.e., is loaded within the nanoparticle). In the second, a cationic polymer is complexed with the genetic material to form a polyplex. Ultimately, the genetic material must be able to cross the cell membrane barrier and be transported into the nucleus to have a therapeutic effect.

3.1. Gene Delivery and MRI. Much like in drug delivery, paramagnetic and superparamagnetic metals have also been used as MRI contrast agents in combination with gene therapy. For example, Wu et al. have prepared a formulation in which DNA bound to a cationic methacrylamide-based polymer that was chelated with Eu^{3+}.[87] Without DNA bound, the Eu^{3+}-polymer compound provided strong contrast in MRI; however, this contrast was diminished upon binding of DNA to the complex. This difference in contrast provided gene delivery information in real time. Bryson et al. utilized another polycation containing either three or four repeating ethylene-amines to package DNA into nanoparticle carriers, thereby protecting DNA from nuclease damage.[88] Gd^{3+} was incorporated into the nanoparticle as well (Figure 8). These polymeric

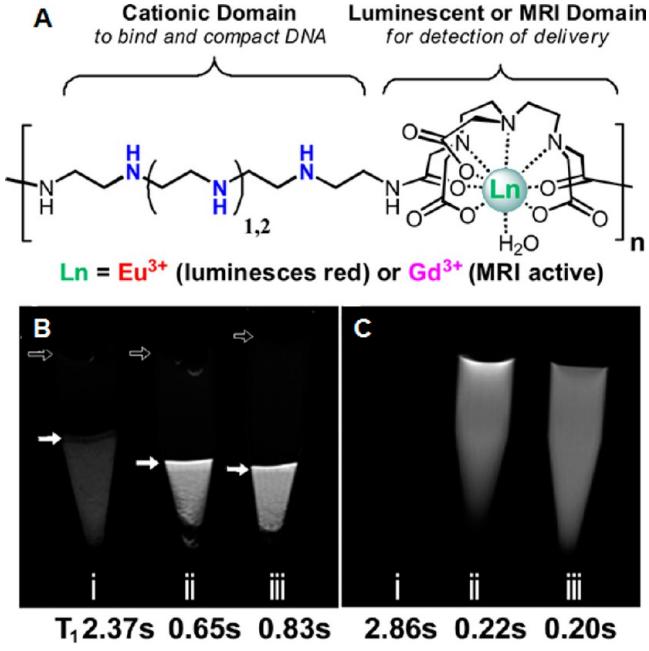

Figure 8. (A) Schematic representation of oligoethyleneamine polymeric beacons conjugated with MRI contrast agents. (B) HeLa cell pellets transfected with Gd3 polyplexes: (i) pellet of untreated HeLa cells; (ii) cells transfected with Gd3a polyplexes; and (iii) cells transfected with Gd3b polyplexes. Solid arrows indicate the buffer-cell interface in each sample. Open arrows indicate perturbations due to bubbles at the buffer-air interface. The darker spots in the cell pellet are due to cell density gradients. (C) MR images of controls without cells: (i) PBS buffer only, (ii) Gd3a only, and (iii) Gd3b only. Gd3a and Gd3b contain three and four ethyleneamines, respectively. Reproduced with permission from reference 88.

delivery vehicles were found to be taken up *in vitro* by human cervix adenocarcinoma (HeLa) cells, and provided contrast at the nanometers/micrometers scale via microscopy and submillimeters scale for MRI.

More commonly used for MRI contrast in theranostics for gene delivery are superparamagnetic systems. Superparamagnetic iron oxide has been used to track PEI-based,[89−92] poly((2-dimethylamino)ethyl acrylate)-based,[93] or poly(propyleneimine) dendrimer-based[94,95] polymeric nanocarriers complexed with DNA or siRNA. Many of these platforms have shown efficacy both *in vitro* and *in vivo* in delivering genetic components to cells and altering gene expression, demonstrating the potential for eventual clinical translation. Combining MRI contrast agents with gene delivery, researchers can better understand the delivery and trafficking mechanisms of gene delivery polyplexes.

3.2. Gene Delivery and Radionuclide Imaging. Radionuclide imaging and gene delivery have not been typically studied together as a theranostic platform. However, Grunwald et al. have demonstrated transfection efficiency using a poly(amidoamine) dendrimer-coated adenovirus to deliver the theranostic sodium iodide symporter (NIS) gene.[96] The dendrimer-coated adenovirus demonstrated an enhanced oncolytic effect following systemic administration. In addition, when expressed by the target cells, NIS causes the accumulation

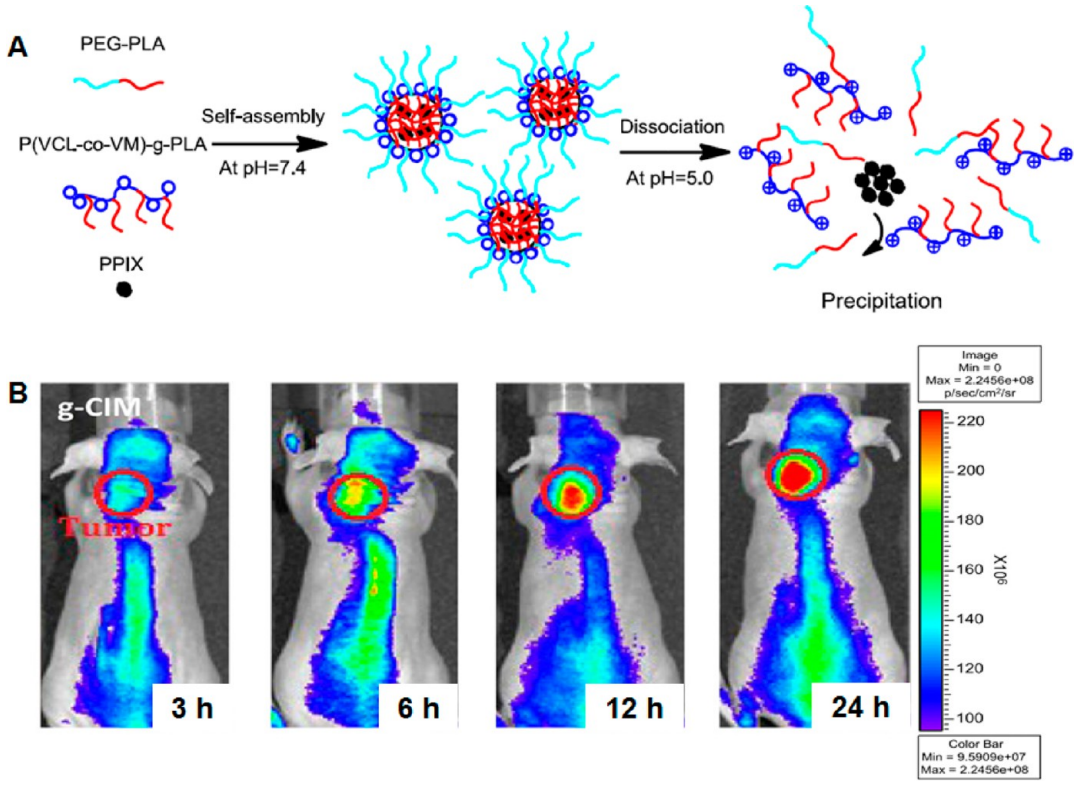

Figure 9. (A) Self-assembly of protoporphyrin IX with poly(*N*-vinyl caprolactam)-*g*-PLA graft copolymer. The polymers self-assemble into micelles at pH 7.4 and encapsulate PPIX, and they disassociate at pH 5.0 to release PPIX. (B) *In vivo* noninvasive fluorescence imaging of A549 tumor xenografted nude mice treated with PPIX-loaded pH-sensitive micelles. The micelles demonstrated high tumor accumulation. Reproduced with permission from reference 126.

of iodine, thereby providing a means for imaging using both two-dimensional ^{123}I scintigraphy and three-dimensional high-resolution ^{124}I PET imaging. In this example, the gene itself acts as the theranostic agent, providing both oncolytic potential and contrast for radionuclide imaging. Gene transfection efficiency can be easily tracked and quantified using this method.

3.3. Gene Delivery and Fluorescence Imaging. The most common approach for image-guided gene delivery is to use fluorescence to track the delivery of genetic material. There are four main categories of fluorescent polymer-based gene delivery systems: (i) DNA complexed with fluorescently labeled polymer, (ii) fluorescent nanoparticle coated with polymer, to which DNA is then complexed, (iii) polymer complexed with fluorescent DNA, and (iv) fluorescently labeled polymer complexed with fluorescently labeled DNA.

In the first category, the polymer itself can be photoluminescent or it can be stained with a fluorescent dye.[97−99] Typically a cationic polymer such as PEI is used to take advantage of electrostatic attraction between the polymer and DNA. In one example, however, Pangburn et al. have developed a noncationic polymersome composed of poly(1,2-butadiene)-*b*-poly(ethylene glycol) diblock copolymers with carboxyfluorescein incorporated.[100] This system was used to deliver siRNA for the knockdown of the Orai3 gene and treatment of breast cancer. By integrating the fluorescent compound into the system, Pangburn and colleagues were able to observe that the polymersome formulation primarily released their cargo in the early endosomal intracellular compartment, and their data suggests that siRNA may be released into the cytosol. This offers a promising start for targeted theranostic delivery of siRNA.

In the second approach, a fluorescent particle is coated with polymer that is associated with DNA or siRNA. QDs are often used for this, and QD-based polymeric delivery is the most common approach for fluorescent polyplex gene therapy. As many other polymer-based gene delivery approaches, cationic polymers are usually used. QDs are coated with amino-containing polymers (i.e., PEI),[101,102] or with a noncationic polymer that has been modified to be positively charged via the attachment of another cationic polymer[103] or tertiary amine groups.[104,105] These new types of siRNA carriers have superior transfection efficiency compared with the traditionally used Lipofectamine, while presenting reduced toxicity. The intrinsic fluorescence of QDs also provides a mechanism for real-time imaging of siRNA delivery both *in vitro* and *in vivo*.

The third method involves labeling of the therapeutic siRNA or DNA component with a fluorescent probe. The siRNA or DNA is typically labeled with a Cy5 dye. Several different polymeric carriers have been investigated in this potential platform, including PAMAM dendrimers,[106,107] PEI,[108,109] poly(glycoamidoamine),[110] and chitosan.[111] By associating fluorescent dye directly with the genetic material cargo, researchers are able to track exactly where DNA or siRNA is localized intracellularly in real time.

In the last category, both the carrier and the therapeutic cargo are labeled with fluorescent agents.[112−116] The two components are typically labeled with probes that fluoresce at different wavelengths. With this method, it is possible to track the fates of each component of the theranostic delivery platform individually upon cellular uptake, thus gaining a better understanding of gene delivery mechanisms and cellular uptake of genetic material-loaded nanocarriers.

4. COMBINED PHOTODYNAMIC THERAPY AND IMAGING

Photodynamic therapy (PDT) achieves its therapeutic effect via a different mechanism from either drug or gene delivery. Rather than delivering an anticancer drug or protein or altering gene expression, nanotheranostic particles used for PDT directly destroy their targets. PDT is a minimally invasive technique that kills target cancer cells in the presence of oxygen via the release of reactive oxygen species upon light activation of a photosensitizer. This destroys cancer cells through direct cellular damage, vascular shutdown, and induction of the host immune response against the target cells.[117] The importance of targeted delivery of the photosensitizer cannot be understated, as the photosensitizer will be activated when exposed to light regardless of its location, leading to potential off-target toxicity. As a result, it is of the utmost importance to be able to monitor PDT agents in real time to reduce the release of reactive oxygen species in healthy tissue areas.

Fortunately, in most cases the photosensitizer used for PDT is also fluorescent, enabling researchers to track the location of the polymeric conjugate. For example, Peng and colleagues have developed a multifunctional polymeric nanoparticle composed of PEG-polycaprolactone diblock copolymer loaded with IR-780, which functions as both a NIR fluorescent dye and a photosensitizer.[118] In BALB/c athymic nude mice bearing HCT-116 colorectal carcinoma, these micellar nanoparticles demonstrated enhanced tumor accumulation and tumor growth inhibition. Chlorin e6 (Ce6) has also been commonly used as a photosensitizer in theranostic applications.[119−121] Lee et al. have used glycol chitosan nanoparticles to load Ce6. Compared with physical encapsulation, chemical conjugation of Ce6 to amphiphilic glycol chitosan-5β-cholanic acid resulted in more sustained release of Ce6, longer circulation half-life, and increased tumor accumulation.[122] NIR imaging of mice bearing HT-29 human colon adenocarcinoma clearly showed strong tumor localization. The subsequent activation of the photosensitizer caused severe tumor necrosis and decreased tumor mass. Porphyrins and their derivatives are another class of photosensitizers that are commonly used.[123−125] Tsai and coworkers have utilized poly(*N*-vinyl caprolactam)-*g*-PLA, a pH-sensitive copolymer with endosomolytic ability, and poly(*N*-vinyl caprolactam-*co*-*N*-vinyl imidazole)-*g*-PLA, a non-pH-responsive copolymer, to encapsulate protoporphyrin IX (PPIX) for *in vitro* and *in vivo* PDT studies (Figure 9).[126] They found that PPIX accumulated in the nucleus of cancer cells when delivered by pH-sensitive particles but was largely trapped in lysosomes when delivered by non-pH-sensitive particles. In an *in vivo* model with mice bearing A549 xenografts, the pH-responsive particles demonstrated better tumor growth inhibition as well, indicating that the interaction between the photosensitizer and tumor cell also affects the efficacy of PDT.

5. CONCLUSIONS

With the ability to provide concurrent therapy and imaging, nanotheranostics have great potential and applicability in medicine and biomedical research. Polymers offer many benefits in their use, including biocompatibility, tailorability, and low cost. They can be used to encapsulate normally insoluble compounds, and they protect their cargo from degradation until they have reached their target location. Of course, there could be concerns about nanoparticle toxicity, as still not much is known about how nanoscale entities behave in human systems. The size and surface properties of nanoparticles, for example, can affect biodistribution and circulation via mechanisms such as nonspecific protein adsorption, macrophage interaction, and disturbance of biological barriers.[127] As an example of the latter phenomenon, highly positively or negatively charged nanoparticles altered the integrity of the blood-brain barrier in rats, while neutral or slightly negatively charged nanoparticles did not.[128] Polymeric nanoparticle formulations are also often relatively polydisperse, making it difficult to thoroughly characterize them to meet regulatory requirements. Additionally, it is possible for the long circulation of polymeric nanocarriers to induce toxicity or hypersensitivity reactions.[129] As a result, careful toxicological testing and analysis is necessary for each new nanostructure. However, researchers are currently engineering increasingly sophisticated architectures with multiple therapeutic and imaging modalities to overcome these limitations.[130]

While theranostic nanoparticles have yet to be utilized in a clinical setting, the considerable advances made in cancer nanotheranostics will likely have far-reaching applications in other important fields such as cardiology[131,132] and tissue engineering.[133] Multifunctional polymeric nanoparticles can enable targeted cancer therapy and imaging and can also facilitate monitoring of the therapeutic effect. However, further *in vivo* work will be required to thoroughly investigate the safety and efficacy of these novel theranostic platforms prior to clinical application. With extensive multidisciplinary cooperation among nanoengineers, bioengineers, materials scientists, biologists, and clinicians, new theranostic nanostructures possess the potential to be translated into the clinic. This new and exciting field has a bright future ahead, offering the unique ability for more personalized medical treatment via simultaneous treatment and monitoring of a plethora of disorders and diseases.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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ABBREVIATIONS

ADR, Adriamycin
Ce6, chlorin e6
CRISPR, clustered regularly interspaced short palindromic repeats
DNA, deoxyribonucleic acid
DOX, doxorubicin
DTX, docetaxel
Gd, gadolinium

Gd-DOTA, gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
Gd-DTPA, diethylenetriaminepentacetate
HPMA, *N*-(2-hydroxypropyl)methacrylamide
ICG, indocyanine green
mPEG-*b*-p(LA-*co*-ME), monomethyl-polyethylene glycol-*block*-poly(lactic acid-*co*-mercaptoethanol)
MRI, magnetic resonance imaging
NIPAAm, *N*-isopropylacrylamide
NIR, near-infrared
NP, nanoparticle
PAMAM, poly(amidoamine)
PCL, polycaprolactone
PDEAEMA, poly(2-(diethylamino)ethyl methacrylate)
PDT, photodynamic therapy
PEG, polyethylene glycol
PEI, polyethylenimine
PEO, poly(ethylene oxide)
PET, positron emission tomography
PGA, poly(*L*-glutamic acid)
PGMA, poly(glycidyl methacrylate)
PLA, polylactic acid
PLGA, poly(lactic-*co*-glycolic acid)
PMAA, poly(methacrylic acid)
PVA, poly(vinyl alcohol)
QD, quantum dot
siRNA, small interfering ribonucleic acid
SPECT, single-photon emission computed tomography
SPIO, super paramagnetic iron oxide

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