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Responsive Theranostic Systems: Integration of Diagnostic Imaging Agents and Responsive Controlled Release Drug Delivery Carriers

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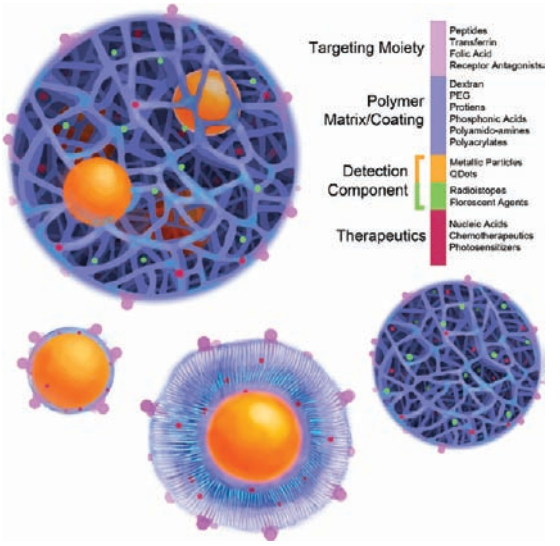
CONSPECTUS

For decades, researchers and medical professionals have aspired to develop mechanisms for noninvasive treatment and monitoring of pathological conditions within the human body. The emergence of nanotechnology has spawned new opportunities for novel drug delivery vehicles capable of concomitant detection, monitoring, and localized treatment of specific disease sites. In turn, researchers have endeavored to develop an imaging moiety that could be functionalized to seek out specific diseased conditions and could be monitored with conventional clinical imaging modalities. Such nanoscale detection systems have the potential to increase early detection of pathophysiological conditions because they can detect abnormal cells before they even develop into diseased tissue or tumors. Ideally, once the diseased cells are detected, clinicians would like to treat those cells simultaneously. This idea led to the concept of multifunctional carriers that could target, detect, and treat diseased cells. The term "theranostics" has been created to describe this promising area of research that focuses on the combination of diagnostic detection agents with therapeutic drug delivery carriers.



Targeted theranostic nanocarriers offer an attractive improvement to disease treatment because of their ability to execute simultaneous functions at targeted diseased sites. Research efforts in the field of theranostics encompass a broad variety of drug delivery vehicles, imaging contrast agents, and targeting modalities for the development of an all-in-one, localized detection and treatment system. Nanotheranostic systems that utilize metallic or magnetic imaging nanoparticles can also be used as thermal therapeutic systems. This Account explores recent advances in the field of nanotheranostics and the various fundamental components of an effective theranostic carrier.

Introduction

Advances in nanotechnology have the ability to significantly influence how diseases are detected and treated. Over the past decade, nanoparticles have been explored for both drug delivery and imaging applications. Nanoparticles are useful in both imaging and therapeutic applications and are exciting for their potential ability to simultaneously image and treat disease at the cellular level. The term "theranostics" has been coined to describe this emerging area of research, which focuses on the combination of diagnostic detection agents with therapeutic drug delivery carriers.

Targeted theranostic nanocarriers present a desirable improvement to disease treatment because of their ability to target and treat specific disease sites while also allowing noninvasive monitoring of particle localization. Research efforts on theranostic carriers encompass a broad variety of chemical constructs; theranostic agents differ greatly in their shape, size, functionality, targeting mechanism, and imaging modality. Despite great chemical diversity among

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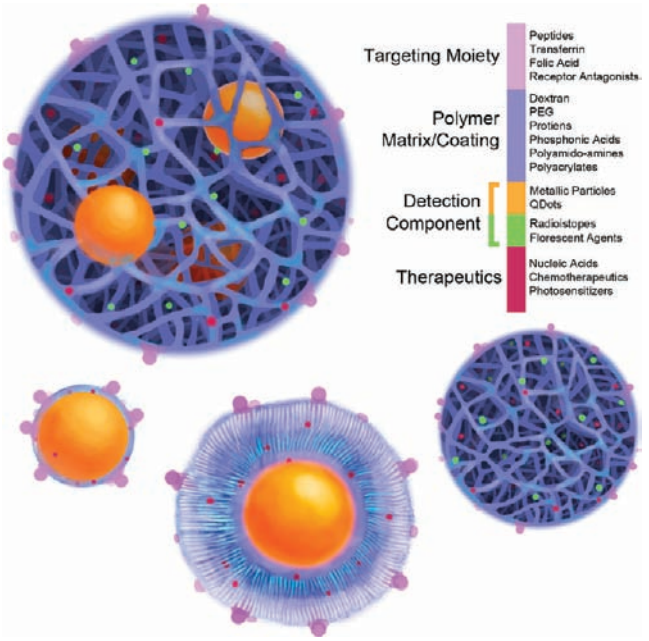

FIGURE 1. Representative theranostic carrier architecture with key components: targeting moiety, therapeutic agents, detection components for noninvasive imaging, and polymer coating or matrix to provide a network for drug loading, impart colloidal stability, and provide functional groups for bioconjugation.

theranostic constructs, several unifying characteristics guide their rational design, as summarized in Figure 1. The archetypal design paradigm for theranostic nanoparticles includes several key components as summarized:

(1) *Detection component for noninvasive imaging*. Imaging agents are typically metallic or magnetic compounds for magnetic resonance imaging (MRI), fluorescent compounds for optical imaging, or radioisotopes for positron emission tomography (PET) or single-photon emission computed tomography (SPECT). Many systems feature a combination of two moieties, though some recent systems possess ternary combinations of these agents for three imaging modalities[1] as shown in Figure 2. By far the most common imaging agent is magnetic iron oxide (IO), which can range in size from <50 nm to 3.5 μm.[2]

(2) *Polymer coating to impart colloidal stability and provide functional groups for bioconjugation*. Magnetic iron oxide particles demonstrate a propensity to aggregate in solution and must be modified with a polymer or surfactant coating to prevent flocculation or aggregation. Depending on the chemical nature of the coating, functional groups such as amines, sulfhydryls, or carboxyl groups can be present for bioconjugation to fluorescent labels or targeting ligands.[3]

(3) *Drug loading capabilities*. Physical encapsulation or entrapment in a polymer matrix, electrostatic interactions with components of the drug delivery system, or covalent conjugation to an imaging agent or nanocarrier are routinely used to impart drug loading capabilities.[1]

In an effort to make disease-specific delivery of contrast agents and therapeutic drugs, advancements in drug delivery systems (DDS) have allowed for controlled release of therapeutic agents in response to their environment, for example, a specific disease condition, pH, or physical inputs like light, temperature, or ultrasound.[4,5] Incorporating contrast agents into DDS allows for the systems' *in vitro* and *in vivo* performance to be monitored. Depending on the contrast agent used, DDS containing contrast agents can be detected using a wide variety of imaging techniques, including optical and nuclear imaging, magnetic resonance imaging (MRI), computer tomography (CT), and ultrasound imaging (US). Theranostic systems can be used to provide noninvasive assessment of carrier biodistribution, quantification of localized accumulation, and drug release. In this Account, we summarize recent research efforts toward the development of nanotheranostic carriers with a focus on key carrier components: responsive polymeric networks for encapsulation of therapeutic agents, imaging agents, some of which can also be used thermal therapy, and targeting agents to enhance localization in a particular tissue or cell type.

Responsive Carrier Systems

Drug delivery systems exhibiting release of therapeutic agents in response to an environmental or external input are generally equipped with polymeric materials that are sensitive to pH,[6] redox potential,[7] temperature,[8,9] or specific biomolecules.[4] In all cases, the stimulus causes physical or chemical changes in the carrier, which lead to release of the encapsulated agents. In the following section, several examples of stimuli-induced drug release in theranostics carriers will be presented.

Early efforts on delivery of therapeutic agents using DDS to specific tissues or cells focused on the treatment of cancer because significant physiological difference between cancerous tissue and surrounding healthy tissue could be exploited. Most of these systems were designed to be pH-responsive to take advantage of the difference in environmental pH between tumors and normal tissue. Depending on the carrier type, anionic or cationic polymers were

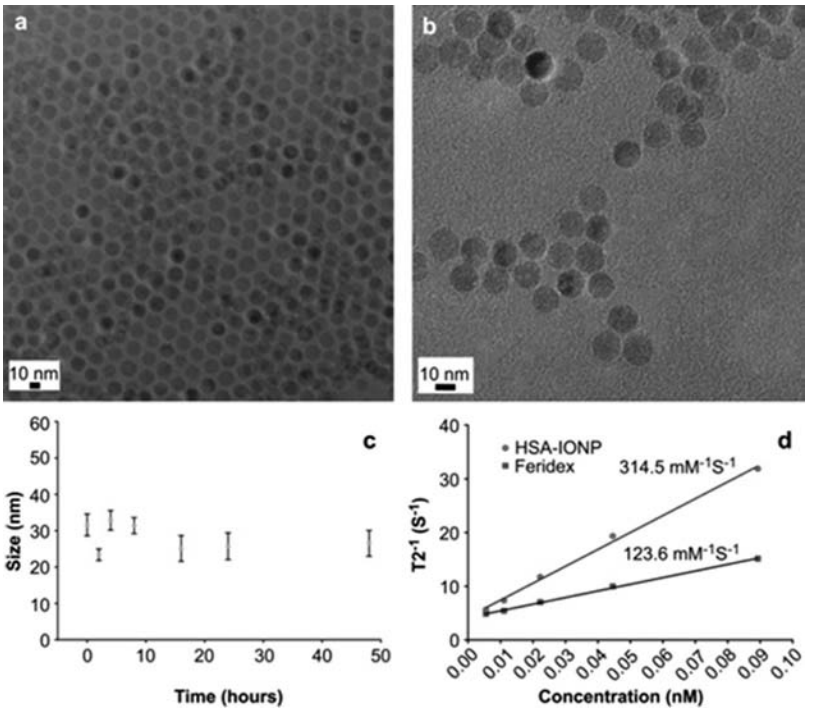

FIGURE 2. Characterization of SPIO-containing theranostic nanoparticles: (a) TEM of oleate-coated iron oxide nanoparticles in hexane; (b) TEM of protein (human serum albumin)-coated iron oxide nanoparticles in water (HSA-IONPs); (c) hydrodynamic size change of the HSA-IONPs when the particles are incubated in PBS at 37 °C for 48 h, monitored by DLS; (d) r_2 relaxivity evaluations with HSA-IONPs and Feridex. Reprinted with permission from Ref.1

incorporated into grafted or block copolymers composed of at least one component that has the ability to ionize at a pH less than physiological pH (7.4). When these homo- or copolymer networks are exposed to the more acidic extracellular environment (pH 6.5−7) associated with tumors, they can undergo changes in physical conformation (swelling and deswelling) in response to thermodynamic factors in the polymer matrix and the surrounding environment. This response can enhance drug transport out of the carrier due to changes in diffusive or convective properties that occur concurrent with swelling or shrinking of the polymer network. Combining materials that exhibit these behaviors with contrast agents allows for pH-responsive theranostic carriers to be developed.

pH-responsive theranostic carriers can also be developed by taking advantage of interactions between the therapeutic agent and polymer network. Therapeutic agents that contain amine groups have demonstrated pH-dependent release; hypothesized to be a result of the amine group protonation.[10] Zou et al.[10] recently reported the development of a novel antibody- and fluorescence-labeled superparamagnetic iron oxide nanoparticle (SPIO) nanotheranostic carrier for MRI and fluorescent imaging and pH-dependent intracellular drug release. To create a dual-imaging particle, the group conjugated the fluorescent dye 5-FAM to the PEG polymer coating on iron oxide nanoparticles (IONPs). Chemotherapeutic drugs containing amine groups were encapsulated within the PEG coating.[10] To facilitate disease-specific targeting, the group conjugated the anti-TAG-72 antibody HuCC49ΔCH2 to the IONPs, which has previously been shown to specifically bind to colon cancer cells *in vitro* and *in vivo*. The group demonstrated that the particles' ability to target and deliver their therapeutic payload to cancerous cells could be monitored using MRI and fluorescent microscopy.[10]

Temperature-responsive polymers have also been explored as platforms for theranostic carriers. Temperature-responsive carriers are typically composed of polymers that have a phase transition in the range of biologically acceptable temperatures (35−42 °C).[9] Changes in physical conformation of polymeric materials (e.g., swelling) that accompany these transitions can be used to release agents physically encapsulated within the matrix. For example, polymers like poly(*N*-isopropyl acrylamide) (PNIPAAm) undergo phase separation as the temperature is increased beyond a critical value, known as the lower critical solution temperature (LCST).

Novel therapeutic nanocomposite systems, where metal nanoparticles like gold[8,9] or SiO_2−gold nanoshells[11] have

been integrated into a temperature-responsive polymer hydrogel network, have also been developed. The metal nanoparticles or nanoshells absorb photons and convert this energy to heat, which causes a localized heating to the surrounding network. Increased temperature causes temperature-sensitive networks to swell or deswell and, therefore, release encapsulated agents.[8,9] Metallic nanoparticles and nanoshells also function as imaging contrast agents, which will be highlighted in the following section.

Detection Components for Noninvasive Imaging and Thermal Therapeutics

Over the past several years, the use of metallic nanoparticles, such as gold and silver, has been a significant development in the field of biomedical research. Evaluation of these nanoparticles as diagnostic agents,[12,13] image-guided drug delivery,[8,11,14] and photothermal therapeutics[13,14] has been demonstrated. The scattering properties of nanostructures, including nanospheres, nanoshells, core–shell particles, and carbon nanotubes allow them to be used for imaging with a wide variety of techniques including optical, confocal, and simple dark field microscopy, MRI, CT, and US. The absorption properties of these nanostrucutures can also be exploited for the benefit of disease treatment. In the following sections, the uses of conductive and semiconductive nanoparticles for (1) evaluation of particle location and biodistribution for potential diagnostic tools and (2) thermal therapeutic tools will be presented.

Diagnostic Imaging. Imaging with contrast agents (both molecular- and nanoparticle-based) is a powerful tool for characterizing biological processes at the cellular and subcellular level, both *in vitro* and *in vivo*. Semiconductor nanoparticle-based contrast agents have multiple advantages over their chemical-based fluorescent and bioluminescent probe counterparts. One of the major advantages is that semiconductor probes do not autobleach and their emission does not decay over prolonged use. Nanoparticles are also well suited for the development of targeted contrast agents because the particle surface serves as a platform for functionalization with targeting molecules and their systemic circulation can be tuned by physical or chemical properties. Depending on the nanoparticle composition, *in vivo* biodistribution can be monitored in real-time through noninvasive imaging. Magnetic particles, like iron-oxide nanoparticles, can be surface-modified with targeting ligands or therapeutic agents and can be monitored with MRI.[3,10]

Coating or embedding contrast agents in polymeric materials is a useful means to increase the circulation time of the contrast agents because polymers can impart colloidal stability and so-called "stealth" properties to the agents. Various constructs have been evaluated including polymer matrices, polymer brushes, dendrimers, micelles, and liposomes. Wiener and colleagues functionalized dendrimers with ion chelate complexes to create viable MRI contrast agent probes.[15] Konda and colleagues have reported the development of a multifunctional polyamido-amine (PAMAM) dendrimer with gadolinium (Gd) chelates and folic acid to target ovarian cancer.[15] Yordanov and colleagues reported the development of a dendritic nanoparticle functionalized with iodine as a CT imaging agent. Liposomal formulations have also been used for carriers of contrast agents including Gd,[15] chelates, radionuclides, iodine-based agents,[15] and quantum dots.

Thermal Therapeutics. Over the past several years, the absorption properties of semiconductive nanoparticles have been explored for therapeutic uses. By adjustment of the shape and size of nanospheres and the shell thickness of nanoshells and core–shell particles, the absorption band of the particles can be tuned to facilitate photothermal therapy within a localized disease region. Photothermal therapy of antibody-conjugated solid gold nanospheres and nanoshells has been shown to selectively target and destroy cancer cells while sparing the surrounding healthy cells.[16] For example, El-Sayed et al. have demonstrated that gold nanorods with an aspect ratio of 3.9:1 have absorption properties within the absorption range of minimum damage to cells and tissues. Upon conjugation of these nanorods with anti-epidermal growth factor receptor (EGFR, a cell-surface receptor overexpressed on malignant cells) antibodies, cancerous cells preferentially internalized the nanorods and were subsequently killed with less than half of the laser energy associated with damage to normal cells.[13]

One major limitation of photothermal therapy is the penetration depth of laser light. Even with near-infrared wavelengths, the light penetration depth is only 2–3 cm, depending on tissue type. This shortcoming limits the use of photothermal therapy with nanoparticles to superficial malignant tumors. Because of this limitation, thermal therapy using other energy sources including radiofrequency, which has excellent tissue penetration *in vivo*, is being investigated.[17]

Radiofrequency ablation (RFA) is a clinical treatment for some malignant tumors including deep tissue tumors in the breast, lung, liver, bone, and kidneys. Unfortunately, current RFA treatment is invasive and requires insertion of a needle electrode directly into the tumor. Additionally, it is nonspecific to malignant cells and therefore leads to damage to surrounding normal tissue. It is limited to certain organs, and RFA treatment does not completely destroy the treated tumor (only 5–40% of

TABLE 1. Selected Examples of Targeted Theranostic Nanoparticles

target	targeting moiety	imaging component	imagining modality	drug	disease model/cell line	ref
$\alpha_V\beta_6$ integrin	peptide sequence (RGDLATLRQL)	SPIO	MRI	doxorubicin	H2009 [lung carcinoma]	20
folate receptor	folic acid	NIR dye, SPIO	fluorescence, MRI	taxol	A549 [lung carncinoma]; H9c2 [cardiomyocyte]	25
CD71 Receptor	transferrin	^{64}Cu	PET/CT	siRNA	neuro2A [neuroblastoma]	26
SIGNR1 Receptor	dextran	SPIO, AlexaFluor 750	MRI, fluorescence	TPC	RAW 264.7 [murine macrophage]; U937 [human macrophage]	28,29
uMUC-1	EPPT	SPIO, Cy5.5	MRI, NIRF	doxorubicin, siRNA	BT-20 [breast adenocarcinoma]	30,31
MMP-2	chlorotoxin	SPIO	MRI	siRNA, DNA	C6 [glioma]	32−34
folate receptor	folic acid	SPIO	MRI	Dox	HeLa	35
folate receptor	folic acid	SPIO, RITC	MRI, fluorescence	MTX	HeLa	36
$\alpha_v\beta_3$ integrin	peptidomimetic receptor antagonist	paramagnetic gadolinium	CRM	fumagillin	atherosclerosis	37

[a]Table abbreviations: MRI, magnetic resonance imaging; Dox, doxorubicin; NIRF, near infrared fluorescence; PET, positron emission tomography; CT, computer tomography; MMP-2, matrix metalloproteinase-2; siRNA, small interfering RNA; TPC, 5-(4-carboxyphenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin; CRM, confocal reflectance microscopy; RITC, rhodamine B isothiocyanate; MTX, methotrexate.

the lesion is destroyed).[17] The use of semiconductor nanoparticles that have the ability to release heat directly to targeted cells and tissue when exposed to radio frequency (RF) field would allow for noninvasive, targeted RF treatment at any site within the body. Curley and colleagues have demonstrated that gold nanoparticles release a fatal dose of heat to cancer cells that have internalized particles when exposed to a focused external RF field.[17] This research has also shown that when these gold nanoparticles are conjugated with cetuximab, they are rapidly internalized by pancreatic adenocarcinoma and colorectal adenocarcinoma cells that overexpress EGFR. In comparison, bare gold nanoparticles are not internalized by these cells. When these cells are then exposed to noninvasive RF fields, close to 100% of the cells are killed.[18]

Targeted Responsive Theranostic Nanoparticles

Both passive and active targeting strategies have been employed in the development of theranostic systems. In passive targeting, nanodrugs can accumulate in solid tumors due to the enhanced permeation and retention (EPR) effect.[19] In actively targeted systems, researchers aim to increase localization at the disease site through specific recognition of certain biomarkers or cellular receptors. This targeting can occur by incorporation of ligands such as peptides,[20] proteins,[21] antibodies,[12,22] aptamers,[23] polysaccharides,[24] or small molecules such as folic acid.[25] It should be noted that previous reports have concluded that the core benefit of targeting strategies lies in the enhancement of cellular uptake in tumor environments rather than any direct influence on *in vivo* biodistribution.[26] Several examples of targeted nanotheranostics are discussed in this section and summarized in Table 1.

Ligands for Theranostic Targeting. Peptides. RGD. Arginine−glycine−aspartic acid (RGD) sequences have been used widely in a number of biomedical fields including biomaterials for tissue engineering and regenerative medicine.[27] RGD fibrinogen, lamin, and collagen of many cell types are known to bind favorably to fibroblasts, endothelial cells, smooth muscle cells, osteoblasts, and chondrocytes[27] through recognition of several different integrins. Researchers in theranostics and nanomedicine have used RGD sequences to increase cellular internalization and impart targeting capabilities to delivery agents.

The $\alpha_v\beta_3$ integrin is a particularly popular target for theranostic agents equipped with RGD sequences. This cell adhesion molecule is richly expressed on invasive tumors, including malignant melanomas and glioblastomas, and is thought to be a key regulator of angiogenesis in tumor sites[38] yet is also present in a panel of other cell types, including macrophages, platelets, lymphocytes, and smooth muscle cells.[39] Mulder et al.[40] describe the PEGylation and subsequent functionalization of quantum dots with RGD peptide sequences and report an increase in cellular internalization of RGD-conjugated quantum dots relative to non-RGD controls. Cyclic RGD peptide sequences have also been used to construct $\alpha_v\beta_3$-targeted nanoparticles for cancer imaging. Other applications include their use in PET/MRI dual-mode imaging[41] and incorporation into ultrasmall (<10 nm) iron oxide nanoparticles.[42]

EPPT. The synthetic peptide EPPT (Cys-$(PEG)_2$-Tyr-Cys-(Acm)-Ala-Arg-Glu-Pro-Pro-Thr-Arg-Thr-Phe-Ala-Tyr-Trp-Gly-Lys(FITC)-$CONH_2$) has been studied as a targeting agent for uMUC-1, an antigen found on greater than 90% of breast adenocarcinomas.[30,31] Using the common cross-linked dextran-coated superparamagnetic iron oxide (SPIO) nanoparticles

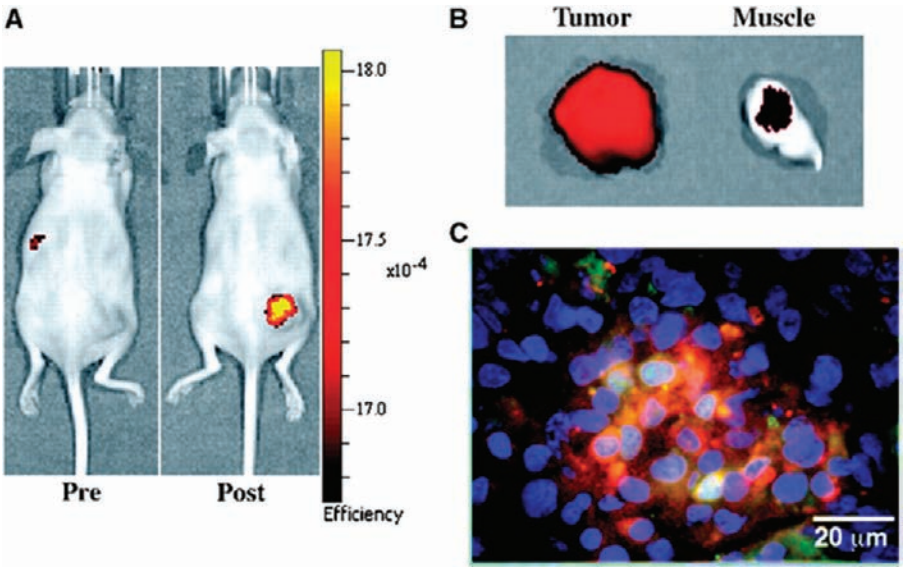

FIGURE 3. Optical imaging in a subcutaneous breast adenocarcinoma tumor model. (A) *In vivo* imaging. The near-IR signal, associated with the tumors compared with surrounding tissue reflected tumor-specific delivery of EPPT-targeted particles carrying siBIRC5. (B) *Ex vivo* imaging. There was a bright near-IR fluorescence associated with the tumors and limited fluorescence of adjacent muscle tissue. (C) Fluorescence microscopy demonstrating colocalization between green fluorescent EPPT (FITC) and red near-IR magnetic nanoparticle (Cy5.5). This reflects the stability of the theranostic particle after persistence in the circulation. Cell nuclei are depicted in blue (DAPI). Reprinted with permission from ref 31. Copyright 2010 American Association for Cancer Research.

as a basis for the delivery vector, Medarova and colleagues constructed a modular theranostic nanoparticle by covalent attachment of Cy5.5 and FITC-labeled EPPT at approximately 2 and 4.5 labels per particle, respectively.[43] This system was designed to provide robust, high-spatial-resolution MR imaging and high-sensitivity optical imaging of tumors *in vivo*. It has been implemented to monitor the response of orthotopic tumor models to chemotherapy[30,43] and also to monitor accumulation and silencing activity of siRNA cargos in implanted tumors.[31,44] In the most recent study, the authors adapted the theranostic nanoparticle to include two layers of tumor specificity incorporating a targeting moiety and therapeutic payload selective for breast adenocarcinomas.[31] The therapeutic payload chosen was small interfering RNA (siRNA) targeting tumor-specific gene *BIRC5,* which encodes an apoptosis inhibitor, survivin. *In vitro* testing of silencing efficacy in BT-20 breast adenocarcinoma cells revealed significant knockdown in *birc5* expression relative to treatment with a scrambled siRNA control. Following systemic injection of targeted, siRNA-laden particles, the authors noted preferential accumulation in the subcutaneously implanted tumor, a significant decrease in the rate of tumor growth, and ultimately a ~40% reduction in tumor volume compared with that in mice treated with scrambled siRNA (Figure 3). Histological analysis, combined with TUNEL assays, revealed mechanisms of cell death included both necrotic and apoptotic pathways. Parallel *in vitro* experiments, particularly those involving silencing efficacy, were conducted with CAPAN-2 (pancreatic adenocarcinoma) and LS-174T (colorectal carcinoma), and they demonstrated robust therapeutic applicability of this theranostic system for imaging and treatment of various cancers.

Transferrin. Targeting the CD71 receptor by surface conjugation of iron-transporter protein transferrin has become a popular strategy in drug delivery. The CD71 receptor or transferrin receptor (TfR) is abundantly expressed in a broad panel of malignant cancer types.[45] Davis and coworkers[26] examined the influence of transferrin-targeted siRNA on biodistribution and resultant silencing activity in neuroblastoma tumors. Their results highlight the importance of complementary detection methods for assessing targeted-therapeutic efficacy. Despite negligible impact on biodistribution relative to nontargeted siRNA nanoparticles, transferrin-targeted particles decreased luciferase activity in the tumor model by nearly 50%. Using a reverse double emulsion technique, Adair and co-workers[46] developed pH-responsive calcium phosphate nanoparticles for sequestration and intratumoral delivery of the insoluble anticancer agent ceramide. The particles, nominally 20−30 nm in diameter, were doped with rhodamine WT and ceramide (C_6 and C_{10}) for simultaneous imaging and induction of apoptosis.

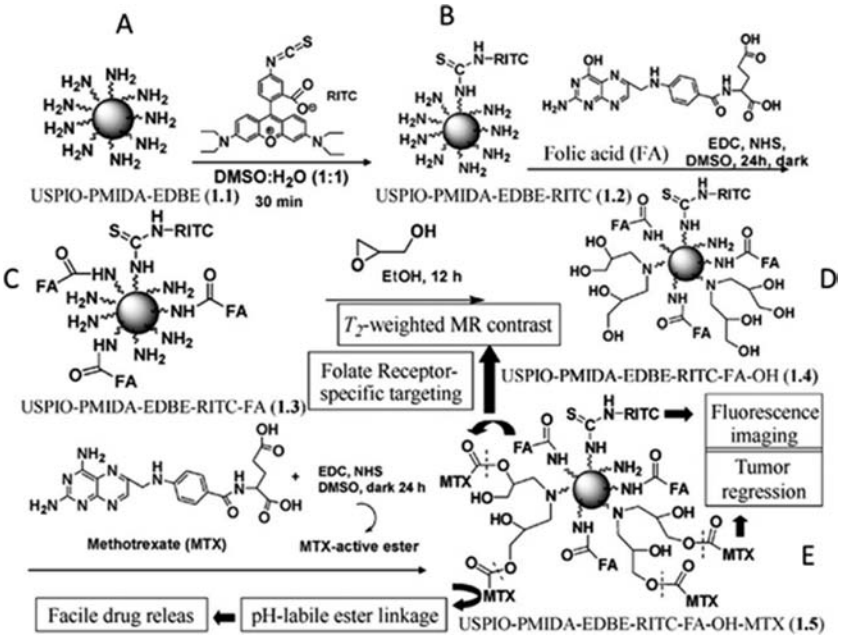

FIGURE 4. Modular construction of multifunctional theranostic agent. PMIDA-coated ultrasmall SPIO (USPIO) nanoparticles are activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) and then reacted with diamine 2,2-(ethylenedioxy)bis-(ethylamine) (EDBE) to create an amine-rich surface for bioconjugation. USPIO−PMIDA−EDBE served as the base material for T_2-weighted magnetic resonance (MR) imaging (A). Next, a fluorescent dye, rhodamine B isothiocyanate (RITC), was conjugated to the USPIO−PMIDA−EDBE surface for fluorescent imaging (B). Third, folic acid (FA) was conjugated to complex B to provide targeting capabilities (C). Last, methotrexate (MTX) was coupled to the nanoparticle surface through a pH-labile ester linkage created by reaction with complex D. The particle contains distinct moieties for targeting, multimodal imaging, and cancer treatment (E). Reprinted with permission from ref 36. Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA.

The control particles (no ceramide) demonstrated minimal toxicity in a melanoma cell model (UACC 903), while the ceramide-doped particles initiated a significant caspase response and caused marked cell death. This particle system was modified to include surface-conjugated human holotransferrin and targeted to the orthotopic tumor model of MDA-MB-231 metastatic breast cancer.[21] In this instance, the authors observed little improvement in tumor accumulation and cellular uptake relative to an untargeted, PEGylated particle.

Small Molecules: Folic Acid. Folic acid has been used in a variety of targeted theranostic systems, including in conjunction with self-assembled doxorubicin (Dox)−PEG conjugates for targeted Dox delivery to KB and A549 xenografts,[47] combined with high-aspect ratio SPIO/Dox polymer vesicles,[35] and in conjunction with gold nanoparticles for targeted delivery of Dox to murine mammary carcinoma cells.[48] A systematic investigation by Li and Yan[49] shed light on the synergistic benefits of multivalent targeting of gold nanoparticles in an epidermal cancer model. The authors reported a marked increase in uptake of targeted particles following conjugation of both folate and glucose to the gold surface. Relative to gold−folate and gold−glucose, the amount of gold per cell (determined by inductively coupled plasma mass spectrometry) increased nearly 4-fold and nearly 13-fold, respectively. Furthermore, the dual-conjugation strategy successfully enhanced uptake selectivity between cells with overexpression (KB) and normal expression (A549) of the folate receptor. Subsequent radiation induced cell-death assays confirmed this result as 80% of KB cells were killed when subjected to X-ray irradiation yet only 15% of A549 cells died. Facile conjugation chemistry, such as "click" chemistry, has given researchers a useful synthetic tool for the rapid development of diverse chemical structures in relatively mild reaction conditions. Santra et al.[25] have used this approach in the development of folate-conjugated theranostic nanoparticles. Particles measured approximately 90 nm diameter in solution, with an 8 nm SPIO shell and 40 nm poly(acrylic acid) coating. Lung carcinoma A549 cells exposed to this construct, loaded with paclitaxel and NIR imaging agent (DiI), internalized the particles, leading to mitotic arrest and cell death. Other chemistries, such as phosphonic acid functionalization, have been explored to enhance colloidal stability and provide sites for bioconjugation on SPIO nanoparticles.[36,50] In one study, Das and Pramanik[36] describe *N*-phosphonomethyl iminodiacetic acid (PMIDA)-coated SPIO, to which rhodamine B isothiocyanate (RITC), folic acid (FA), and methotrexate (MTX) were surface-conjugated, as shown in Figure 4. Cellular internalization of this trifunctional nanoparticle was much greater in HeLa cells (folate receptor +) than in MG-63

(folate receptor −) and apoptosis was observed in HeLa cells following exposure to particles, indicating liberation of MTX from the particle surface.

Synthetic Receptor Antagonists. Lanza and colleagues have devoted efforts toward targeting and imaging sites of angiogenesis, which can serve as a key indication for tumor formation or development of artherosclerotic plaques. This group has worked extensively to develop targeted nanoparticles for the $\alpha_v\beta_3$ integrin. Using a peptidomimetic vitronectin antagonist, perfluorocarbon nanoparticles were targeted to Vx-2 tumors and imaged through incorporation of high-sensitivity radionuclides[39] such as ^{111}In[51] and ^{99m}Tc. Examination of the tumor/muscle contrast ratio revealed a significant contrast ratio enhancement (6.3 ± 0.2 for ^{111}In and 8.56 ± 0.13 for ^{99m}Tc) generated by targeted radioactive particles in tumor neovasculature relative to the surrounding muscle tissue. A distinct advantage of radionuclide imaging is that small tumors can be identified rapidly using robust scanning techniques with a wide field of view. In a series of studies Winter et al.[37,52,53] have employed targeted theranostics as a mechanism for detecting and inhibiting blood vessel growth in atherosclerotic plaques. Several factors, including oxidative stress, hypoxia, and stimulatory growth factors are thought to promote neovasculature formation in plaque regions.[37] In a promising study, Winter et al.[37] demonstrated the prolonged antiangiogenic effect of a combination therapy consisting of $\alpha_v\beta_3$-targeted fumagillin nanoparticles and atorvastatin. Initially, paramagnetic nanoparticles loaded with antiangiogenic agent fumagillin were injected into New Zealand white rabbits on a high-cholesterol diet. The targeted particles alone were able to decrease markers of neovascularization over 50% by 1 week, and the signal suppression, indicative of decreased angiogenesis, was sustained for 4 weeks. In a subsequent experiment, the combination of $\alpha_v\beta_3$-targeted fumagillin nanoparticles and atorvastatin was evaluated for its ability to maintain suppression of angiogenesis in the aorta. Cardiac magnetic resonance (CMR) imaging demonstrated that both control (hyperlipidemic) rabbits and those receiving dietary atorvastatin alone demonstrated identical angiogenic signals, suggesting that atorvastatin did not significantly impact plaque angiogenesis over the time scale of this experiment (results shown in Figure 5). However, rabbits receiving two doses (0 weeks and 4 weeks) of $\alpha_v\beta_3$-targeted fumagillin nanoparticles and dietary atorvastatin exhibited a marked decrease in CMR signal from aortic neovasculature. The authors concluded that the synergistic therapeutic benefit of $\alpha_v\beta_3$-targeted fumagillin nanoparticles and dietary atorvastatin was far greater than that of the two treatments independently and that acute antiangiogenic effects previously demonstrated by fumagillin[53] were prolonged by combination with atorvastatin.

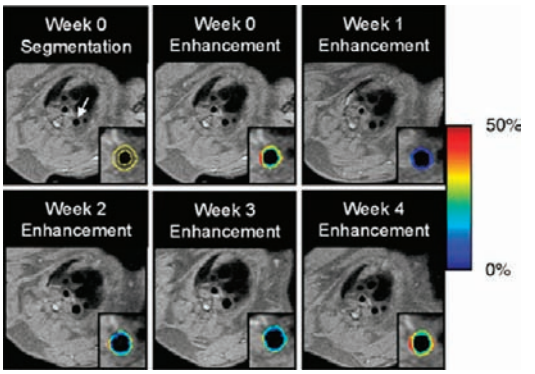

FIGURE 5. Segmentation of the aortic wall and color-coded signal enhancement before and after targeted fumagillin treatment (top). Black blood image of the thoracic aorta (arrow) and segmentation of the vessel wall (outlined in yellow) is shown for the week 0 image. The color-coded overlay of signal enhancement (%) shows patchy areas of high angiogenesis. On the week 1 image, the signal enhancement has clearly decreased due to the antiangiogenic effect of targeted fumagillin treatment. The level of signal enhancement gradually increases at weeks 2 and 3 after fumagillin treatment (bottom), until week 4, when the level of enhancement is practically identical to the week 0 image. Reprinted with permission from ref 37. Copyright 2008 Elsevier.

Conclusion

Targeted, dual functional delivery of diagnostic and therapeutic agents is a significant unmet need in the field. Despite tremendous effort in targeted carriers and directed drug delivery systems, true targeting remains elusive. While surface conjugation of various peptide sequences, proteins, antibodies, and other small molecules can effectively enhance the preferential uptake of nanocarriers at disease sites, this enhancement is largely probabilistic (i.e., more receptors per unit area increases the chance of receptor−ligand interaction and subsequent internalization). Given adequate circulation time, the current generation of nanotheranostics will ultimately traffic to highly perfused tissue such as the liver, lungs, pancreas, kidneys, tumors, and sites of angiogenesis.

The next generation of intelligent delivery systems should be deterministic rather than probabilistic; capable of tunable pharmacokinetics and biodistribution whereby the carriers will localize at, and only at, the intended disease targets and in response to the diseased environment will release their therapeutic payload. In addition, they should be able to seek, find, and persist in disease sites while providing concomitant treatment and noninvasive monitoring.

The ultimate goal is to develop systems that can tailor treatment to a specific individual by localized release at the specific pathophysiological site. We seek to develop systems

that provide health professionals the ability monitor disease states and either trigger appropriate release of a therapeutics or monitor stimuli-responsive release. This form of treatment leads to higher patient survival and better quality of life by effecting optimized treatment at a disease site while minimizing potential adverse effects.

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BIOGRAPHICAL INFORMATION

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FOOTNOTES

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