

The Upcoming Field of Theranostic Nanomedicine: An Overview

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Nanocarriers have drastically changed the face of health care by making a mark in diverse arenas of diagnosis, drug delivery, and gene delivery to name a few. The recent feat in nanotechnology has been the birth of nanotheranostics which aims at blending both therapeutic and diagnostic functions within a single nanoscaffold. The field of theranostic nanomedicine is a result of fruitful advances in fields of material science, imaging modalities, formulation development, and molecular biology. Theranostic nanomedicine that was at first developed for enhancing the quality of treatment meted out to cancer patients has now been explored even in atherosclerosis and infections, albeit to a lower extent. The review summarizes various types of nanocarriers that have been explored with one or sometimes multiple imaging modalities for an array of applications ranging from drug delivery and gene delivery to photosensitizing agent delivery for photodynamic therapy. The article also highlights the few but significant developments made in the field of theranostic nanomedicine for atherosclerosis and infections. In conclusion, theranostic nanomedicine is a rapidly growing field. However, there are a few problems that need to be addressed before theranostic nanocarriers carve a niche for themselves in the clinic.

Keywords: Theranostic, Imaging, Fluorescence, Nanocarrier, Quantum Dots, Gold.

CONTENTS

1. Introduction	859
2. Imaging Modalities	860
2.1. MRI	860
2.2. FI	860
2.3. Ultrasound (US) Imaging	861
2.4. Radionuclide Imaging (RI)	861
3. Nanocarriers	861
3.1. Liposomes	861
3.2. Micelles	863
3.3. Dendrimers	863
3.4. Nanoparticles	863
3.5. Carbon Nanotubes	863
4. Cancer Theranostics	863
4.1. Simultaneous Imaging and Chemotherapeutic Delivery	864
4.2. Simultaneous Imaging and Gene Delivery	869
4.3. Simultaneous Imaging and Photothermal Therapy	871
4.4. Combined Imaging and Photodynamic Therapy	873
5. Theranostic Agents for Atherosclerotic Lesions	875
6. Theranostic Agents for Infection	876
7. Conclusion	876
Acknowledgments	877
References and Notes	877

1. INTRODUCTION

Nanotechnology is a burgeoning field today driven by the incredible support received from miscellaneous disciplines of academia, industry and federal agencies. Nanotechnology has significantly revolutionized drug therapy with the advent of nanosized drug delivery systems like liposomes, dendrimers, micelles, nanoemulsions, polymeric nanoparticles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), self nanoemulsifying drug delivery systems, nanocrystals, carbon nanotubes, and ceramic nanoparticles. Benefits of nanoparticles in drug therapy include their ability to be targeted specifically to disease sites and their potential to deliver molecules exhibiting unfavourable physicochemical characteristics such as poorly soluble drugs.¹ Also, nanotechnology based diagnostics have already made remarkable advancement with the dawn of several nanoscale imaging platforms in market and some others which are under preclinical or clinical investigation. Nanoparticles are promising platforms for development of contrast agents because they are endowed with a surface which is amenable for modification with one or more targeting moieties at a broad series of densities to selectively image a specific area of interest, also their plasma circulation time can be adjusted based on

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their physicochemical properties, and contrast agents can be incorporated at preset ratios either in the core or on the surfaces.²

The latest buzz in the field of nanomedicine is theranostics. Theranostics is a concept that merges therapeutics with diagnostics to develop new customized treatments with augmented efficacy and safety. The rationale behind the birth of theranostics was the infinitely heterogeneous nature of cancer which invariably renders all the commonly employed treatments effective in only a minor population of patients and at particular phases of disease progression. Theranostics was created with the anticipation that a blend of diagnosis and therapeutics would usher in therapeutic protocols that are more individual-specific.³

Since its inception, the field of theranostics has been constantly growing with a plethora of research groups venturing majorly into theranostics for cancer and to a lesser extent for other diseases such as infections and cardiovascular diseases. The review summarizes the various imaging modalities used for diagnosis, various nanocarriers used in drug delivery, and recapitulates active research efforts to combine these that have led to the birth of numerous theranostic nanocarriers for different diseases such as cancer, atherosclerosis and infections.

2. IMAGING MODALITIES

Numerous imaging modalities have been developed till date each of them having their own advantages and limitations. Imaging modalities commonly employed include Magnetic resonance imaging (MRI), fluorescence

imaging (FI), ultrasound (US) imaging, and radionuclide imaging (RI). Table I highlights the benefits, drawbacks and commercial applications of these imaging modalities.

2.1. MRI

MRI is a non-invasive imaging technique which uses a strong magnet and radiofrequency waves to produce images of internal organs. Colloidal iron oxide nanoparticles (IONPs) such as superparamagnetic iron oxides (SPIO) and ultrasuperparamagnetic iron oxides (USPIO) have been the most widely explored magnetic nanoparticles (MNPs) for biomedical applications attributable to their exceptional biocompatibility and ease of synthesis.⁴ Moieties having high affinity to area of interest have been conjugated to MNPs to enable visualization with better specificity.

2.2. FI

In vivo FI employs a sensitive camera to detect fluorescence emitted from fluorophores in living organisms.⁵ The near infra-red (NIR) region offers decreased autofluorescence, decreased scattering by tissue and greater penetration depth favourable for *in vivo* imaging.² Probes used for FI include fluorescent dyes such as Indocyanine green (ICG), Fluorescein isothiocyanate (FITC), Alexa Fluor 750, Cy5.5; gold nanoparticles or nanorods and quantum dots (QDs). QDs are semiconductor nanocrystals about 2–10 nm in diameter. The most widely used QDs for biological purposes include cadmium selenide, cadmium telluride, indium phosphide, and indium arsenide.⁶ QDs



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Table I. Benefits, drawbacks, and commercially available imaging agents of various imaging modalities.

Imaging modality	Benefits	Drawbacks	Commercially available agents
MRI	High spatial resolution and no exposure to harmful radiations ²	Poor sensitivity ²	Lumiren®, Gastromark®, Feridex IV®, Endorem®, Combidex® (USPIO based in late stage clinical trials) ⁴
FI	High sensitivity in picomolar range, non-invasive, inexpensive, and no exposure to ionising radiation ²	Poor spatial resolution, poor tissue penetration (0–2 cm), highly vulnerable to noise ²	
US	Less expensive, non-invasive, safe, simple, fast, ² availability of portable hand-held US units, and availability of 3D transducers to capture whole beating heart at 30 frames per second in real time ¹⁴	Erratic attenuation by the body tissues in obese patients, general failure of US to image lung, and relatively small size of the field of view in case of the standard 2-dimensional transducer ¹⁴	Optison®, ¹⁵ Definity, ¹⁴ Levovist™ ¹⁶
RI		Exposure of contrast agent formulator, physician, and patient to radioactive material, does not give any anatomical details, and limited temporal resolution of SPECT ¹³	18-fluorodeoxyglucose, ¹⁷ ¹²³ I-meta-iodobenzylguanidine, ¹⁸ ^{99m} Tc pertechnetate ¹⁸

are ideal probes for real time monitoring of cellular interactions *in vivo* because of their superior fluorescence as compared to organic fluorophores (10–100 times brighter), resistance to photo bleaching (100–1000 times more) as compared to organic fluorophores, and long blood circulation time.^{7,8} QDs possess 10–50 times larger molar extinction coefficients compared to organic dyes, and as a result are brighter than the latter in photon-limited *in vivo* conditions. Further, their emission wavelengths are amenable to tuning by changing their size. Larger particles emit light in the red region of the visible spectrum, and smaller particles emit in the blue range.⁸ QDs are also amenable to modification with suitable ligands via electrostatic interactions, covalent bonding, and adsorption depending on the ligand in question.⁹ However, a few drawbacks still hinder their widespread use, one of them being the nephrotoxicity of cadmium. One can encapsulate QDs in biodegradable polymers so as to prevent cadmium release *in vivo*.¹⁰ Another problem is the quenching of fluorescence of QDs upon contact with aqueous solutions. Coating QDs helps to overcome the quenching effect.⁶ Gold nanoparticles or nanorods are better probes for FI than QDs and organic fluorescent dyes as they are non-toxic or less toxic as compared to QDs, and do not undergo quick photo bleaching like organic fluorescent dyes.¹¹

2.3. Ultrasound (US) Imaging

US technique employs a transducer emitting sound waves having high frequency of approximately greater than 20 kHz. Microbubbles used as contrast agents are composed of a core made up of gases such as octafluoropropane, decafluorobutane, and sulphur hexafluoride; and a shell which may be of a lipid, polymer or albumin.² When the gas microbubbles are exposed to US, echoes are created from which images of internal organs are produced.¹²

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2.4. Radionuclide Imaging (RI)

Radionuclide imaging techniques are Single Photon Emission Computerized Tomography (SPECT) and Positron Emission Tomography (PET). Radionuclides which emit gamma rays are used in SPECT and a gamma camera rotates 360 degrees around the patient to obtain several images from different positions. Radionuclides typically employed for SPECT include ⁶⁷Ga, ^{99m}Tm, ¹¹¹In, and ¹²³I having long half lives of 3.3 days, 6 hours, 2.8 days, and 13.1 hours respectively. PET employs radionuclides which undergo decay by emitting positrons which interact with electrons in the body to produce a pair of annihilation photons which are detected. Majority of the radionuclides employed in PET have short half lives such as ¹¹C (20 min), ¹³N (10 min), and ¹⁵O (2 min). PET cameras possess approximately 50–100 times greater sensitivity of detection and 3–4 fold greater spatial resolution as compared to SPECT.¹³

3. NANOCARRIERS

Numerous nanocarriers have been developed in recent years using a diverse array of materials each offering different attributes in terms of biocompatibility, drug loading, and stability. The following section gives a glimpse of the various nanocarriers that have been developed in recent years. Figure 1 gives a pictorial representation of different theranostic nanocarriers. Table II enlists the benefits, drawbacks, and applications of various nanocarriers.

3.1. Liposomes

Liposomes are lipidic vesicles encapsulating an aqueous core. Liposomes are mainly composed of phospholipids—either natural or synthetic. Commonly used natural

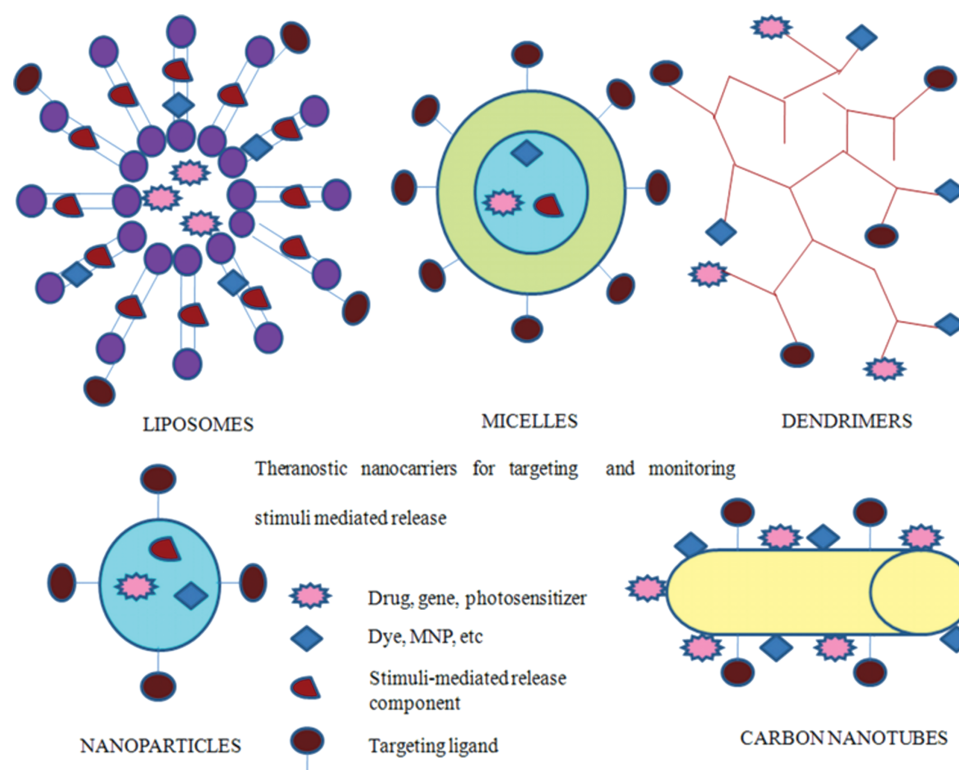


Fig. 1. Pictorial representation of different types of theranostic nanocarriers.

phospholipids include phosphatidylcholine, phosphatidylglycerol, and phosphatidylethanolamine.¹⁹ Commonly used synthetic phospholipids include Dilauryl phosphatidyl choline, Dimyristoyl phosphatidyl choline, Dipalmitoyl phosphatidyl choline, Distearoyl phosphatidyl choline, Dioleoyl phosphatidyl choline, Dilauryl phosphatidyl ethanolamine, Dimyristoyl phosphatidyl ethanolamine, Distearoyl phosphatidyl ethanolamine,

Table II. Benefits, drawbacks, and applications of nanocarriers.

Nanocarrier	Benefits	Drawbacks	Application
Liposomes	Biocompatibility, biodegradability, ability to modify drug distribution <i>in vivo</i> , and ability to protect drug from degradation ²⁷	Low stability, poor batch to batch reproducibility, poor loading capacity, and difficulties in sterilization ²⁷	Doxil, Ambisome, DaunoXome, DepoDur ²⁸
Micelles	Low CMC (10^{-6} – 10^{-7} M) leading to prolonged circulation <i>in vivo</i> and small size, both leading to EPR effect, high loading capacity, biodegradability, active targeting, and smart micelles which release drug in response to stimuli like temperature, pH, and US waves ^{21, 29}		Genexol TM 6
Dendrimers	High drug loading capacity due to multivalent architecture, active targeting, and ability to decide excretion mode in mammals (urinary versus bile) as a function of nanoscale diameter (generation level) ³⁰		PolyFect (gene delivery) ³¹ Photodynamic and photothermal therapy ²⁴ Delivery of Paclitaxel (PTX), Methotrexate, and Doxorubicin ²⁴
SLN	Ease of manufacture, biocompatibility, possibility of controlled release, amenability to surface modification, and protection of drug from degradation ³²	Drug expulsion ³²	
CNTs	Offer multiple attachment sites for functionalization with moieties, ² exhibit strong optical absorbance in NIR region allowing their use in photothermal therapy ³	Non-biodegradable nature, lack of standard procedure for large scale preparation of highly pure CNTs ³	Delivery of PTX, ³³ Cisplatin, ^{34, 35} DNA, ^{36, 37} and siRNA ^{38, 39}

Dioleoyl phosphatidyl ethanolamine, Dilauryl phosphatidyl glycerol, and Distearoyl phosphatidyl serine. Cholesterol may also be included in liposomes. It functions as a fluidity buffer. Liposomes can be classified based on their method of preparation, composition, and structural parameters as depicted in Figure 2.²⁰

3.2. Micelles

Polymeric micelles (PM) are composed of an inner core formed by the hydrophobic portion of the block copolymer (which houses the drug); and an outer shell formed by the hydrophilic block of the copolymer which shields the drug from the aqueous milieu and, by decreasing interactions with opsonins it stabilizes the PM against capture *in vivo* by the reticuloendothelial system (RES). The hydrophobic core is commonly composed of poly(*L*-amino acid), polyesters, and Pluronics. Widely used poly(*L*-amino acids) are poly(*L*-aspartate) and poly(*L*-glutamate). Some of the most commonly used polyesters are poly(glycolic acid), poly(*D*-lactic acid), poly(*D,L*-lactic acid), copolymers of lactide/glycolide, and poly(ϵ -caprolactone).²¹ Also, electrostatic interactions between charged block copolymers and oppositely charged macromolecules results in the formation of “polyion complex” (PIC) micelles which are useful for the delivery of small interfering RNA (siRNA).²²

3.3. Dendrimers

Dendrimers are defined as “core-shell nanostructures with fixed architecture and low polydispersity, which are produced in a layer-by-layer manner (expressed in ‘generations’) around a core unit, culminating in high level of control over size, branching points and surface

functionality.”²³ The most commonly studied dendrimer is Polyamidoamine dendrimer (PAMAM), other dendrimers under investigation are poly(propyleneimine) (PPI), poly(glycerol-co-succinic acid), poly(*L*-lysine), poly(glycerol), poly(2,2-bis(hydroxymethyl)propionic acid) and melamine dendrimers. Gadolinium (Gd) paramagnetic contrast agents for MRI have been complexed with dendrimer molecules for contrast enhancement and better clearance characteristics.²⁴

3.4. Nanoparticles

Nanoparticles are colloidal carriers having size in the range of 1–1000 nm fabricated either from lipid or polymer. The drug may be encapsulated within the nanoparticle core, conjugated or adsorbed onto the surface of nanoparticles. Nanoparticles are also amenable to surface modification with ligands for active targeting. Poly lactide-co-glycolide (PLGA), Poly ϵ -caprolactone, poly lactic acid (PLA), poly glycolic acid (PGA), and poly glutamic acid are the commonly used polymers to fabricate nanoparticles since their biocompatibility is well established. SLN comprise nanoparticles made from a solid lipid or a blend of solid lipids. Another class of lipidic nanocarriers are NLC which include a blend of solid and liquid lipids resulting in better drug loading and avoiding drug expulsion problem associated with SLN.²⁵

3.5. Carbon Nanotubes

Carbon nanotubes (CNT) are defined as “rolled up seamless cylinders of graphite sheets, showing supreme physical, chemical, and mechanical properties.”²⁶ They are further classified as single walled carbon nanotubes (SWCT) (1–2 nm diameter) or multiwalled carbon nanotubes (MWCT) (10–100 nm diameter) depending on the number of graphite layers which make up the nanotube structure. CNT are highly hydrophobic rendering them water insoluble and hence surface modification becomes obligatory to make them water soluble as well as to make them less toxic and biocompatible.²⁶

Theranostic nanocarriers have been explored in cancer, and in atherosclerosis and infections to a lesser extent.

4. CANCER THERANOSTICS

Cancer continues to remain the major cause of mortality worldwide. Commonly used treatment modalities such as chemotherapy, radiation, and surgery are usually associated with problems such as nonspecific distribution of drug resulting in undesired exposure of non target tissue to drug, insufficient concentrations of drug reaching the target site, and lastly restricted ability to monitor therapeutic response.⁴⁰ Theranostic nanocarriers help to overcome these problems as they permit delivery of

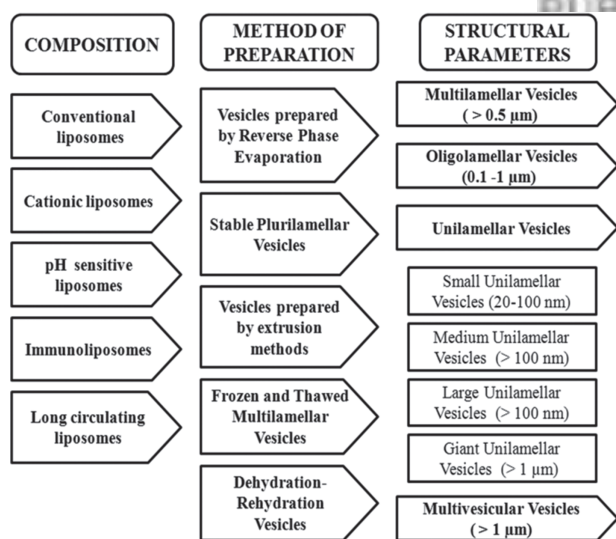


Fig. 2. Classification of liposomes based on composition, method of preparation and structural parameters.²⁰

therapeutic agents coupled with simultaneous monitoring of their release. Also, by incorporating a targeting moiety, the theranostic nanocarrier can be specifically delivered to site of action allowing targeted therapy and diagnosis.

Theranostic nanocarriers have been extensively explored in the area of cancer treatment using various imaging modalities and drug delivery systems in four ways as shown in Figure 3.

4.1. Simultaneous Imaging and Chemotherapeutic Delivery

Different types of theranostic nanocarriers have been employed for simultaneous imaging and delivery of chemotherapeutics. They are classified into following eight categories depending on whether they provide unimodal or bimodal imaging, their targeting potential, and whether the release of drug is stimuli mediated or not.

4.1.1. Theranostic Nanocarriers Providing Unimodal Imaging and Drug Delivery

Yang et al. developed endosome escapable poly(amino acid) nanoparticles loaded with IO nanocrystals and Dox, and conjugated with histidine moiety on the surface. Ionization of histidine moiety in the acidic pH of endosome resulted in endosomal escape of nanocarrier and release of drug at acidic pH. The nanocarriers exhibited superior cytotoxicity than unconjugated nanoparticles loaded with Dox.⁴¹ Cittadino et al. developed paramagnetic liposomes containing an amphiphilic gadolinium complex (present in lipid bilayers) and prednisolone phosphate. Incorporation of an amphiphilic contrast agent enhances the contrast and enables MRI detection at low or intermediate magnetic field strengths of 0.2–1.5 T. The nanoplatform permitted non-invasive monitoring of biodistribution in mice bearing melanoma B16 syngeneic tumour.⁴² Dox loaded

QD-liposome hybrid vesicles (100–120 nm) were developed by Tian et al. QDs were included in the lipid bilayers. Fluorescence of both Dox and QDs was retained. The study was the first one of its kind to fabricate drug containing liposome-QD hybrid vesicles in the size range of 100–120 nm endowed with both imaging and drug delivery functions. However, the cell killing efficacy and pharmacokinetics of the system needs to be evaluated.⁴³ Feng et al. prepared conjugated nanoparticles by electrostatic interaction of cationic conjugated polymer PFO (polyfluorene containing oxadiazole moieties) with anionic poly(*L*-glutamic acid) conjugated with anticancer drug Dox. Dox quenches the fluorescence of PFO by electron transfer mechanism, and hence PFO is in the fluorescence “turn-off” state. Once, the nanoparticles are exposed to carboxypeptidase or are taken up by a cancer cell, poly(*L*-glutamic acid) is hydrolysed in lysosome to release the Dox, provoking the start of PFO fluorescence to “turn-on” state. The multifunctional nanoparticulate system could perform dual functions of drug delivery to cancer cells and imaging based on fluorescence signal of PFO.⁴⁴

Conjugated polyelectrolytes (CPE) obtained by conjugating charged side chains to conjugated polymers are promising fluorescent tracers because of their low cytotoxicity and brighter fluorescence than molecular dyes. Ding et al. first prepared a molecular brush comprising a CPE backbone onto which polyethylene glycol (PEG) chains were attached. Cisplatin was incorporated by complexation of platinum of cisplatin with terminal carboxylic acid group of PEG. The nanoprobe demonstrated near infra red fluorescent (NIRF) visualization of HepG2 cells *in vitro*, real time NIRF imaging in nude mice, and sustained release of cisplatin.⁴⁵ Porous silicon offers several attractive attributes for fabrication of theranostic scaffolds such as adjustable pore size and volume, simple chemical functionalization to incorporate drugs, large surface area and biocompatibility. Wu et al. incorporated Daunorubicin into particles of porous Si photonic crystals through covalent conjugation. Optical reflectivity of porous photonic crystals endows the system with the capacity to monitor drug release. The nanoprobe shows a distinct intense reflectance at a particular wavelength which changes as drug release occurs. Depletion of drug molecules from the scaffold or degradation of silicon matrix leads to decrease in the refractive index of silicon layers and shift from red to blue colour. The nanoprobe demonstrated sustained drug release at therapeutic concentrations for 30 days. The nanoprobe also demonstrated enhanced anti-proliferative effect relative to that of only drug injection and at lower concentrations than those used in drug injection.⁴⁶

Table III enlists a few more theranostic nanocarriers that have been explored for unimodal imaging and drug delivery.

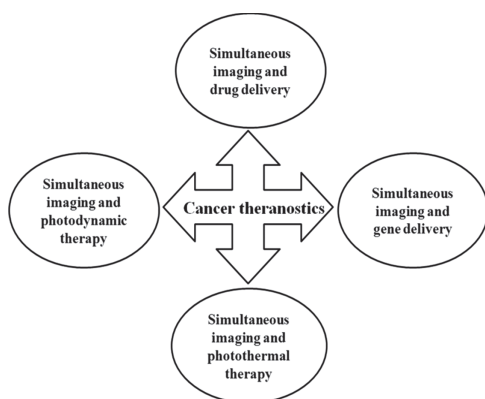


Fig. 3. Schematic representation of different types of theranostics for cancer.

Table III. List of theranostic nanocarriers explored for unimodal imaging and drug therapy.

Nanocarrier	Imaging moiety	Drug	Key features	Ref.
Poly(aspartic acid) nanoparticles	IO nanocrystals	Dox	High cytotoxicity against SKBR-3 cells and two fold higher relaxivity than commercial product	[47]
Oleic acid coated IONPs	IONPs	Dox and PTX	Fivefold longer half life than Feridex IV™ and sustained release of both drugs for three weeks	[48]
Mesoporous nanocapsules comprising silica shell and IO core	Fe ₃ O ₄	Dox	Superior cytotoxicity than free Dox against MCF-7 cell line and good MRI capability both <i>in vitro</i> and <i>in vivo</i>	[49]
Hybrids of MWCT and cobalt-ferrite	Cobalt-ferrite	Dox	Sustained and pH responsive release of Dox at acidic pH, low cytotoxicity, hemolytic activity and excellent MRI enhancement	[50]
Chitosan nanoparticles	Cy5.5	PTX	Enhanced therapeutic efficacy and reduced PTX toxicity in tumour bearing mice	[51]
QD-conjugated CNTs	QD-conjugated CNTs	PTX	Strong luminescence for optical imaging and high loading efficiency for PTX	[52]
Mesoporous lanthanide oxide (Gd ₂ O ₃ :Eu) nanoparticles	Lanthanide oxide (Gd ₂ O ₃ :Eu)	Dox	Optical imaging, sustained drug release and potent cytotoxic activity against HeLa cells	[53]

4.1.2. Targeted Theranostic Nanocarriers Providing Unimodal Imaging and Drug Delivery

The nanocarriers are endowed with targeting potential by functionalization with targeting moieties such as aptamers, peptides, folic acid, antibodies, etc. for specific binding to cancer cells, thus preventing the non specific distribution of drug and imaging moiety and thereby improving the theranostic efficacy of nanocarriers.

Zhang et al. developed polycarboxybetaine methacrylate (PCBMA) nanogels (110 nm) by cross linking PCBMA monomers using a disulfide cross linker. Nanogels were loaded with dextran as a sample drug and Fe₃O₄ nanoparticles for MRI, and functionalized with cRGD peptide for targeted imaging and delivery. The nanogels exhibited targeted delivery to human umbilical vein endothelial cells. Once the nanogels enter the intracellular reductive zone, the disulfide bonds are reduced thus releasing dextran and imaging moiety.⁵⁴ Guo et al. developed multifunctional nanocarriers of Adriamycin by coating Fe₃O₄ cores with mixture of the triblock copolymer methoxy PEG-*b*-poly(methacrylic acid-*co*-*n*-butyl methacrylate)-*b*-poly(glycerol monomethacrylate) and the folate-conjugated block copolymer folate-PEG-*b*-poly(glycerol monomethacrylate). Drug loading was done at pH 7.4 via ionic bonding and hydrophobic interactions. Protonation of polycarboxylate ions of methacrylate in acidic conditions (pH less than 5.5) prevailing in the endosome/lysosome resulted in release of the drug from the carrier due to ionic bond cleavage. Folate conjugation improved the cellular uptake and hence therapeutic efficacy significantly in folate receptor overexpressing HeLa cells relative to the non-targeted carriers.⁵⁵ Li et al. developed micelles comprising a branched polyester core, inner hydrophobic polycaprolactone layer, and a hydrophilic oligo (ethylene glycol) monomethyl ether methacrylate

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(OEGMA)-Gd-folic acid corona. Micelles loaded with PTX showed controlled release of drug, good MRI contrast *in vitro* and *in vivo*, and enhanced cytotoxic effect against HeLa cells *in vitro*.⁵⁶

Bagalkot et al. prepared QD-aptamer-Dox (QD-Apt-Dox) conjugate for targeted imaging and therapy. A10 RNA aptamer was first covalently attached to the QDs. QD-Apt conjugate was then incubated with Dox to form QD-Apt-Dox conjugate. The intercalation of Dox within the double stranded aptamer results in quenching of fluorescence of QDs by Dox and quenching of fluorescence of Dox by aptamer via Bi-Fluorescence Resonance Energy Transfer (FRET) mechanism. Once, the conjugate binds to Prostate Specific Membrane Antigen (PSMA) on prostate cancer cells and enters the cells, Dox is gradually released from the conjugate. Dox release triggers the activation of fluorescence of both QDs and Dox which aids in monitoring drug release.⁵⁷ Santra et al. developed folic acid targeted theranostic nanoparticles using hyperbranched polyhydroxyl polymer. Amphiphilic cavities in nanoparticles were useful to encapsulate both hydrophilic protein cytochrome C and amphiphilic dye ICG. Release of cytochrome C inside the cytoplasm resulted in commencement of apoptosis and subsequently programmed death of folate receptor positive human lung carcinoma A549 cells.⁵⁸ Heparin causes killing of cancer cells via induction of apoptosis. Lee et al. fabricated gold nanoparticles onto which fluorescent dye tagged heparin was immobilized for optical detection of metastasis and also conjugated the same with RGD peptide for targeted cancer cell death. FRET took place and the detachment of heparin from the nanocarrier by heparinase enzyme (over expressed in metastatic cancer cells) resulted in recovery of fluorescence of dye for visualization of heparin release.⁵⁹ Nurunnabi et al. prepared conjugates of PEG with PCDA (10,12-pentacosydonic

acid) and PCDA with Herceptin. These conjugates were formulated along with QDs to get micelles. The micelles exhibited superior anti-tumour efficacy as compared to Herceptin alone. The micelles demonstrated 77.3% tumour growth reduction in athymic BALB/c-nu/nu female nude mice bearing HER-2-positive MDA-MB-231 tumours as compared to control group.⁶⁰ Muthu et al. fabricated TPGS (*D*-alpha-tocopheryl polyethylene glycol 1000 succinate mono-ester) coated folic acid conjugated theranostic liposomes (210 nm) comprising Docetaxel and QDs by solvent injection technique. TPGS coating was done in order to achieve long circulation and improved cellular uptake. The theranostic nanoplatform not only demonstrated no burst release and sustained release for more than one day, but also showed 97.58% reduction in IC₅₀ value as compared to Taxotere® after 24 hours of incubation with MCF-7 breast cancer cells.⁶¹

Table IV enlists a few more targeted theranostic nanocarriers that have been explored for targeted unimodal imaging and drug delivery.

4.1.3. Theranostic Nanocarriers Providing Dual Modal Imaging and Drug Delivery

These nanocarriers are endowed with multiple imaging agents in order to couple the advantages of different imaging modalities. Both MRI and FI are used together to achieve good spatial resolution as well as high sensitivity respectively. Lee et al. developed dye-doped mesoporous nanoparticles containing Dox within the pores and magnetite nanocrystals were immobilized on the surface. The system thus was capable of dual monitoring of drug delivery via MRI and FI.⁸¹ Choi et al. fabricated 3D poly(propylene fumarate) (PPF) scaffolds with Dox and IONPs by mixing IONPs containing Dox with the scaffold. The nanocarriers were capable of both MRI, and optical imaging due to Dox and showed sustained drug release. These nanoscaffolds hold great promise for tumour theranostics due to their biocompatibility, large surface area for high drug loading, and adjustable degradation rate.⁸² Chen et al. fabricated hydroxyapatite nanorods doped with europium (Eu³⁺) and gadolinium (Gd³⁺) ions containing Ibuprofen (model drug) using the time and energy saving microwave assisted hydrothermal method. The nanoprobe provided sustained release of Ibuprofen, demonstrated low cytotoxicity, and *in vitro* MRI and *in vivo* photoluminescence imaging and CT imaging.⁸³

4.1.4. Targeted Theranostic Nanocarriers Providing Dual Modal Imaging and Drug Delivery

These nanocarriers are endowed with multiple imaging agents in order to couple the advantages of different imaging modalities and also a targeting moiety is incorporated with the aim of achieving specificity against cancer cells.

Park et al. developed multifunctional nanoparticles containing Dox, IONPs, and quantum dots within PEG modified phospholipid micelles for optical imaging, MRI and drug delivery. F3 peptide which binds to the nucleolin present on the endothelial cell surface of tumour blood vessels was used for targeting. Cells treated with targeted nanoparticles showed greater fluorescence and MRI contrast as compared to non-targeted nanoparticles.⁸⁴ Santra et al. developed a biocompatible bimodal theranostic nanoplatform comprising of IO nanocrystals coated with Polyacrylic acid (PAA). A NIR dye and Taxol were encapsulated in the hydrophobic spaces of PAA and the nanocarriers were functionalized with folic acid for targeting. The nanoplatform combined three functions of targeted drug delivery, MRI and optical imaging.⁸⁵ Liong et al. encapsulated superparamagnetic IO nanocrystals inside mesoporous silica nanospheres and labelled them with FITC. Folic acid was used as targeting ligand and PTX was loaded into the pores. The nanocarriers exhibited increased specificity towards cancer cells.⁸⁶ Cho et al. developed a spherical polystyrene matrix to which QDs were conjugated and Fe₃O₄ nanoparticles were embedded in the matrix. PTX was loaded onto the surface by using PLGA layer and nanocarriers were functionalized with anti-PSMA. The study demonstrated enhanced targeting to tumour cells *in vitro* and *ex vivo*.⁸⁷ Abdalla et al. demonstrated six fold enhancement in cytotoxic efficacy against prostate cancer cells compared to free noscapine using targeted nanoparticles loaded with IO and Cy5.5 and functionalized with urokinase plasminogen activator receptor ligand. Noscapine was adsorbed onto the amphiphilic polymer coating and at lower pH (4) the hydrogen bonding between drug and polymer was reduced resulting in release of drug.⁸⁸

4.1.5. Theranostic Nanocarriers Providing Unimodal Imaging and Stimuli Mediated Release

In these nanocarriers, drug release is mediated in response to some stimuli like pH, temperature, etc. or drug delivery is guided using a magnetic field. The aim is to limit drug release to the desired site and not throughout the body for enhancing therapeutic efficacy and reducing side effects.

Wu et al. designed a theranostic nanoplatform for simultaneous FI and dual temperature and pH responsive delivery of Temozolomide. They incorporated CdSe QDs within hydroxypropylcellulose-poly(acrylic acid) semi-interpenetrating polymer networks. The nanogels demonstrated pH responsive drug delivery and sustained release of drug.⁸⁹ Kiewit et al. prepared Dox containing superparamagnetic iron oxide nanoparticles (SPIONs) by covalent linkage of Dox to Polyethyleneimine (PEI) via a pH responsive hydrazone bond and to IONPs coated with amine terminated PEG. Dox was released from the nanoparticles at acidic pH of 4–5 similar to the pH

Table IV. List of targeted theranostic nanocarriers explored for unimodal imaging and drug therapy.

Nanocarrier	Imaging moiety	Drug	Targeting moiety	Key features	Ref.
Micelles	SPIO	Dox	Lung cancer peptide	Highly sensitive MRI, superior cytotoxic efficacy compared to non-targeted micelles	[62]
Micelles	SPIO	Dox	cRGD	Ultrasensitive MRI and targeted delivery to $\alpha_v\beta_3$ positive tumour cells	[63]
SPIONs	SPIONs	Dox	cRGD	Selective binding and greater cytotoxicity against $\alpha_v\beta_3$ positive cells compared to non targeted SPIONs	[64]
PEG-poly(<i>D,L</i> -lactide) vesicles	SPIONs	Dox	Folate	Enhanced cytotoxicity due to targeted delivery	[65]
PLGA nanoparticles	SPIO	Docetaxel	Single chain prostate stemcell antigen antibodies	Sustained drug release, enhanced uptake and anti-proliferative activity against PC3 cell line	[66]
Mesoporous silica nanospheres	MNP	Docetaxel	Folic acid	Targeted cytotoxicity against cells overexpressing folate receptor	[67]
IONPs	IO	Methotrexate	Chlorotoxin	Tumour specific MRI and increased cytotoxicity	[68]
PEG-poly ϵ -caprolactone micelles	SPIO	Dox	Folic acid	Targeted theranostics to Bel 7402 hepatic carcinoma cells	[69]
PLGA nanoparticles	Fe ₃ O ₄	Dox	Herceptin	Ultrasensitive MRI and sustained drug release for three weeks	[70]
IONPs	IO	Dox	Transferrin antibody	Sustained release and 2 fold reduction in IC ₅₀ of drug loaded nanoprobes due to targeting	[71]
Glycerol monooleate coated IONPs	IO	PTX, Rapamycin	HER2 antibody	Sustained release for more than 2 weeks <i>in vitro</i> and potent anti-proliferative activity against MCF-7 cells	[72]
SPIONs	SPIONs	Dox	A10 RNA aptamer binding to PSMA	Selective MRI and drug delivery to PSMA expressing prostate cancer cells <i>in vitro</i>	[73]
Liposomes	Gadolinium	Dox	Neural cell adhesion molecule	Enhanced efficacy and reduced toxicity of drug	[74]
Carboxymethyl chitosan-manganese doped zinc sulphide nanoparticles	QDs	5-Fluorouracil	Folic acid	Binding to breast cancer cell line and controlled release of drug	[75]
Fluorescent polydioctyl-fluorenebenzothiadiazole-bisthiophenyl benzothiadiazole copolymer nanoparticles	FI	Herceptin	Herceptin	Selective growth inhibition of HER2 overexpressing tumour cells	[76]
<i>N</i> -succinyl- <i>N</i> -octyl chitosan (SO-chitosan) micelles	Fluorescein	Not used	Folic acid	Drug needs to be incorporated to estimate anti tumour efficacy, targeting efficiency established	[77]
Micelles having core of poly(HEMA-co-histidine)-g-PLA and a shell of PEG-PLA	Cy5.5	Dox	Folic acid	pH dependent drug release at endosomal pH due to histidine groups and enhanced cellular uptake due to targeting	[78]

Continued.

Table IV. Continued.

Nanocarrier	Imaging moiety	Drug	Targeting moiety	Key features	Ref.
Gold nanoparticles	Dox	Dox	PSMA specific aptamer	Targeted theranostics for prostate cancer	[79]
Yb and Eu doped NaYF ₄ upconversion nanoparticles	Upconversion luminescence imaging	Dox	Folic acid	Targeted imaging and cytotoxicity against KB cells	[80]

encountered in endosomal/lysosomal compartments thus protecting it from efflux mechanism. Dox uptake and retention did not differ among wild type and the resistant type cells when nanoparticles were administered, whereas free drug treatment resulted in greater uptake and retention in wild type cells as compared to resistant ones. Also, the resistant type cells were more responsive to nanoparticle conjugated Dox as compared to free drug.⁹⁰ Wu et al. designed covalently cross-linked chitosan-poly(methacrylic acid) (PMMA) nanogels containing CdSe QDs capable of simultaneous imaging and pH responsive drug delivery. The researchers first designed chitosan-PMMA semi-interpenetrating network nanogels followed by *in-situ* synthesis of CdSe QDs within the nanogels. Temozolomide was loaded onto the hybrid nanogels via hydrogen bonding of the amide groups in temozolomide with the hydroxyl and carboxylic groups present in the hydrogel network. Drug release was sustained from the hydrogel network as compared to free drug solution at low pH values of 5–7.4 encountered in tumour extracellular region.⁹¹

Kono et al. prepared multifunctional liposomes providing temperature responsive drug release of Dox and MRI detection. They loaded poly(2-ethoxy(ethoxyethyl)vinyl ether) chains with a lower critical solution temperature around 40 °C and PAMAM G3 dendron-based lipids having Gd³⁺ chelate residues into PEGylated liposomes. Liposomal treatment coupled with local heating to 44 °C for 10 minutes inhibited tumour growth in mice more efficiently as compared to local heating and liposomal treatment employed individually.⁹² Langereis et al. developed temperature sensitive liposomes containing Dox and gadolinium complex for simultaneous MRI and temperature responsive drug delivery. The study demonstrated rapid release of drug and contrast agent at 42 °C.⁹³ Tagami et al. developed thermo-responsive liposomes made of dipalmitoylphosphatidylcholine and Brij 78, containing Dox and gadolinium complex for temperature sensitive release of Dox in a locally heated tumour environment and simultaneous monitoring of the same through MRI. The nanocarriers exhibited 100% drug release within 3 minutes on heating to 40–42 °C.⁹⁴

Sanson et al. prepared vesicles using poly(trimethylene carbonate)-poly(L-glutamic acid). Maghemite nanoparticles and Dox were loaded into the membrane of the vesicles for simultaneous MRI and magnetic field

guided controlled release of Dox.⁹⁵ Gao et al. developed polymeric copolymer stabilized perfluoropentane (PFP) nanoemulsion systems. On heating to physiological temperatures, PFP (boiling point 29 °C) vaporizes inside the bubble walls resulting in the formation of nanobubbles. A small number of microbubbles are also formed due to coalescence of nanobubbles. Dox was loaded in the bubble walls stabilized by the copolymer. Application of tumour directed US resulted in cavitation of microbubbles and collapse resulting in release of the drug only in tumour regions. The microbubbles also provided excellent selective US contrast in tumour regions. The study demonstrated effective chemotherapy of MDA MB231 breast cancer tumours using this system.⁹⁶ Rapoport et al. developed mixtures of micelles of Dox and PFP microbubbles which provided high US contrast and US mediated release of Dox. Exposure to US radiation resulted in cavitation of microbubbles and hence enhanced cellular uptake of the same. US is also supposed to aid in intracellular uptake of nanocarriers by disruption of membrane integrity.⁹⁷

4.1.6. Targeted Theranostic Nanocarriers Providing Unimodal Imaging and Stimuli Mediated Release

Kohler et al. prepared IONPs covalently conjugated to methotrexate by a linkage that is cleaved only in the acidic environment of the lysosome. This nanoplatform not only allowed for real time monitoring of drug delivery via MRI but also reduced the toxicity of methotrexate by limiting drug release to the lysosome after internalization of nanocarrier into folic acid receptor expressing tumour cells. The nanoparticles exhibited similar cytotoxic efficacy as soluble Methotrexate in serum in HeLa and MCF-7 cell lines.⁹⁸ Savla et al. developed QD-mucin-1 aptamer-Dox conjugates for targeted imaging and pH responsive drug delivery for ovarian cancer. Mucin-1 is known to be mutated and overexpressed in 90% of late stage epithelial ovarian cancers. Dox was conjugated to QDs via a hydrazone linkage that was stable at neutral pH values and degraded at acidic pH. Targeted conjugates showed enhanced uptake by multidrug resistant ovarian cancer cell line A2780/AD relative to the non-targeted conjugates and were found to be more toxic as compared to free Dox.⁹⁹

4.1.7. Theranostic Nanocarriers Providing Dual Modal Imaging and Stimuli Mediated Release

Rapoport et al. have developed perfluorocarbon nanoemulsions of PTX for bimodal imaging using MRI and US, and US mediated drug delivery. Perfluoro-15-crown-5-ether (PFCE) droplets were stabilized using Polyethylene oxide-Polycaprolactone (PEG-PCL) and Polyethylene oxide-Poly(*D,L*-lactide) (PEG-PDLA) block copolymers. Twenty equivalent fluorine nuclei present in PFCE resulted in a sharp fluorine peak in the MR spectra and permitted ^{19}F MRI of PFCE nanoemulsion both *in vitro* and *in vivo*. The nanoemulsions exhibited long circulation *in vivo*, MRI and US visualization and good tumour regression activity and suppression of metastasis in breast and pancreatic cancer animal models.¹⁰⁰ Wu et al. developed multifunctional nanogels loaded with 5-Fluorouracil consisting of a bimetallic Ni–Ag core surrounded by a shell consisting of copolymer gel of poly(ethylene glycol-co-methacrylic acid). The nanocarriers provided both pH responsive MRI and fluorescent detection, and drug delivery. The porous structure of nanogels further makes them efficient carriers for drug release.¹⁰¹

4.1.8. Targeted Theranostic Nanocarriers Providing Dual Modal Imaging and Stimuli Mediated Release

Das et al. developed a theranostic nanoplatform integrating two imaging modalities namely MRI and optical imaging and also providing targeted delivery and pH triggered drug release at site of action. They first coated USPIO core with biodegradable *N*-phosphonomethyl iminodiacetic acid (PMIDA) coating. Rhodamine B isothiocyanate, folic acid and methotrexate were coupled to the amine derivatized USPIO-PMIDA support as a fluorescent dye, targeting moiety and anticancer drug respectively.¹⁰² Yang et al. developed a theranostic nanoplatform for targeted multimodal imaging and pH responsive drug delivery. They prepared SPIONs followed by tagging with ^{64}Cu , functionalization with *c*-RGD peptide and conjugation with Dox. Dox was conjugated to the SPIONs via a pH responsive hydrazone linkage. *c*-RGD peptide provided targeted delivery to $\alpha_v\beta_3$ integrin expressing tumour cells. The nanoparticles (size 68 nm) exhibited high tumour accumulation in tumour bearing mice and also provided MRI relaxivity comparable to that of Feridex.¹⁰³ Zou et al. developed theranostic nanoprobe demonstrating dual FI and MRI, and targeted delivery of anticancer drugs to LS174T colon cancer cells followed by pH mediated intracellular release of the drug. Researchers coated SPIONs with oleic acid and amphiphilic polymer followed by PEGylation. They also conjugated Tumour Associated Glycoprotein-72 targeting antibody and a fluorescent dye to carboxyl groups of PEGylated SPIONs encapsulating Dox. Targeted nanocarriers exhibited IC_{50} value of

0.44 μM which was significantly lower than that of the non-targeted nanocarriers (1.42 μM).¹⁰⁴

4.2. Simultaneous Imaging and Gene Delivery

Gene delivery involves delivery of genetic material such as DNA or siRNA into the host cell to replace a mutated gene causing disease with a healthy copy of the same gene, for deactivating or “knocking out,” a mutated gene that is functioning improperly, and for introducing a new gene into the body to help fight a disease. siRNA is a short double-stranded RNA containing about 19–23 nucleotides. siRNAs cause degradation of complementary mRNA at a specific sequence by means of RNA interference. siRNAs have found wide application in malignancies, autoimmune conditions, and infectious diseases attributable to their superior target gene silencing capacity. However, the delivery of siRNAs is hindered by its speedy degradation in circulation by nucleases, poor intracellular uptake and failure of endosomal escape.¹⁰⁵

Researchers have explored a wide variety of vectors for gene delivery, both viral and non-viral. Non-viral vectors show less immunogenic and cytotoxic potential as compared to viral ones, but their low transfection efficiency relative to viral ones poses a hindrance to their application. Non-viral vectors explored include cationic polymers, lipids, and cationic liposomes.¹⁰⁶ Nanocarriers have several favourable attributes such as size, shape and possibility of surface modification that qualify them as promising gene delivery platforms. There is a need for real time imaging of siRNA transport into the cell and subsequent release at site of action.

Researchers have created numerous theranostic nanocarriers capable of simultaneous gene delivery and imaging. They can be classified into four categories based on whether they provide unimodal or dual modal imaging, and based upon their targeting potential.

4.2.1. Theranostic Nanocarriers Providing Unimodal Imaging and Gene Delivery

Bhakta et al. formulated gadolinium oxide doped silica nanoparticles followed by surface modification with 3-aminopropyltrimethoxysilane to bind DNA through electrostatic interaction. The nanoprobe demonstrated 75% and 77% *in vitro* transfection efficiencies relative to Polyfect in COS-7 and 293T cells respectively.¹⁰⁷ Feng et al. developed a fluorescent nanocarrier based on lipid functionalized cationic polymer poly(fluorenylene phenylene). The lipid was biocompatible and protected the conjugated polymer chain. Ammonium pendant groups provided sites for attachment of plasmid DNA (pDNA) encoding green fluorescent protein (GFP). The nanocarriers exhibited good photostability and low cytotoxicity. The nanocarriers (size 50 nm) were uniform and

demonstrated successful transfection in A549 lung cancer cells.¹⁰⁸ Qi et al. developed nanoprobe using QDs and amphipols for simultaneous siRNA delivery and imaging. Amphipols are linear polymers composed of alternate hydrophilic and hydrophobic side chains. The researchers employed poly(maleic anhydride-alt-1-decene) modified with dimethylamino propylamine to form complex with QDs via hydrophobic interactions. At neutral pH, the structure had positive charge which enabled electrostatic binding of siRNA and interaction with negatively charged membranes of cells. The tertiary amine cluster on the surface resulted in proton absorption in acidic endosome leading to osmotic swelling and bursting of endosome. The carboxylic groups in polymaleic anhydrides also aid in proton absorption. The study demonstrated gene delivery potential of the nanoprobe in both serum free and serum containing media in contrast to Lipofectamine which is only effective in serum free medium. The nanoprobe showed superior gene silencing as compared to PEI in serum containing and serum free media, protected siRNA from degradation by nucleases, and the toxicity of QDs was reduced because amphipol layer prevented interaction of cadmium with the intracellular milieu.¹⁰⁹ Li et al. developed *L*-arginine and beta-cyclodextrin-*L*-Arginine functionalized CdSe/ZnSe QDs loaded with siRNA (through electrostatic binding to positively charged surface of modified QDs) for silencing HPV18E6 gene in HeLa cells. These QDs showed low cytotoxicity, offered protection to naked siRNA against nuclease and demonstrated superior gene silencing activity (more than 80%) in HeLa cells as compared to commercial transfection agents. β -cyclodextrin-*L*-Arginine functionalized QDs were found to be superior in terms of both siRNA protection and gene silencing, the former attributed to steric hindrance and the latter to increased water solubility and biocompatibility favouring higher cellular uptake.¹¹⁰ Similarly, the same group developed *L*-Histidine- β -cyclodextrin functionalized QDs loaded with siRNA for silencing HPV18E6 gene in HeLa cells, and demonstrated gene silencing activity comparable to that of commercial transfection agents.¹¹¹ Shim et al. synthesized a theranostic nanoplateform by covalently coating siRNA encapsulating polyplexes with small gold nanoparticles by acid-cleavable linkages. Within the mildly acidic tumour environment, the gold nanoparticles were cleaved from the surface resulting in a signal followed by the exposure of the polyplex having positive surface charge and small size which facilitates its high intracellular uptake. The study demonstrated stimuli sensitive imaging and gene delivery using a single nanoplateform.¹¹²

4.2.2. Targeted Theranostic Nanocarriers Providing Unimodal Imaging and Gene Delivery

Cheong et al. incorporated SPIONs into the core of the nanoparticles formed from conjugates of water soluble

chitosan with linoleic acid. These SPION loaded nanoparticles were complexed with *p*DNA or siRNA for GFP. The authors demonstrated hepatocyte specific delivery due to linoleic acid moiety, and, enhanced gene expression (DNA) or silencing (siRNA) both *in vitro* and *in vivo*.¹⁰⁶ Bae et al. prepared hollow manganese oxide nanoparticles (HMONs) and surface functionalized them with PEI-3,4-dihydroxy-*L*-phenylalanine (DOPA) conjugate, Herceptin was also used as a targeting moiety. HMONs having cationic shell formed stable polyelectrolyte complexes owing to the electrostatic interactions of the negatively charged phosphate groups in siRNA with the positively charged amine groups of PEI. This stabilized siRNA against enzymatic degradation in presence of fetal bovine serum for more than 50 minutes as compared to 15 minutes in case of naked siRNA. The nanoparticles were found to specifically detect and exert gene silencing effect in HER-2 over expressing cancer cells.¹⁰⁵ Guihua et al. developed a theranostic non-viral polymeric vector for diacylglycerol kinases gene delivery to *T* cells for immunosuppression which are generally tough to transfect. CD3 single chain antibody was conjugated onto the distal ends of PEG-grafted PEI as a targeting moiety for *T* cells. This modified polymer was then complexed with SPIONs and then *p*DNA was incorporated. The nanocarriers demonstrated 38% inhibition in IL-2 expression thus indicating arrest of *T* cell proliferation and 43% inhibition in proliferation of rat *T* lymphocyte cells.¹¹³

Jiang et al. developed silica coated NaYF₄ upconversion nanoparticles doped with lanthanide ions, followed by conjugation with folic acid and anti-HER2 antibody. The nanocarriers provided targeted two-photon FI and effective silencing of luciferase gene.¹¹⁴ In another study, Bartlett et al. conjugated DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to siRNA followed by labelling with ⁶⁴Cu and transferrin conjugation for active targeting to tumours. PET imaging showed enhanced intracellular localization of siRNA and thereby better gene silencing effect in case of targeted nanoparticles as compared to non-targeted nanoparticles.¹¹⁵

Table V lists some more nanocarriers that have been explored for monitoring targeted gene delivery.

4.2.3. Theranostic Nanocarriers Providing Multimodal Imaging and Gene Delivery

Medarova et al. developed magnetic nanoparticles tagged with Cy5.5 dye and linked to myristoylated polyarginine peptides for cytosolic translocation of complexes with anti-survivin siRNA. Survivin is a protein abundantly expressed in tumours and not in normal cells, thus making it an ideal target for cancer therapy. The study demonstrated dual MRI and NIRF imaging and good target gene silencing in tumour bearing mice.¹²¹ Kenny et al. have designed theranostic PEGylated siRNA nanoparticles called Lipo-some Entrapped siRNA nanoparticles. siRNA used was

Table V. List of targeted theranostic nanocarriers explored for unimodal imaging and gene delivery.

Nanocarrier	Imaging moiety	Gene	Targeting moiety	Key features	Ref.
IONPs coated with PEI	IO	GFP gene	Chlorotoxin	2.5 fold increase in gene knockdown as compared to non-targeted	[116]
IONPs coated with copolymer of chitosan, PEG and PEI	IO	DNA encoding GFP	Chlorotoxin	Increased GFP expression due to specific uptake of targeted nanocarrier in C6 glioma cells in xenograft mouse model	[117]
QDs	QDs	siRNA for EGFP	Tumour targeting peptide F3	Substantial knockdown of EGFP signal in EGFP transfected HeLa cells	[118]
PAMAM dendrimer	QDs	Vimentin shRNA plasmid and yellow fluorescent protein siRNA	Epidermal Growth Factor (EGF)	Targeted imaging and delivery in EGF receptor positive cells over negative cells	[119]
Bifunctional Au/Ni nanorods	Au	GFP and luciferase reporter gene	Transferrin	Targeting resulted in two fold greater GFP expression and 3.4 fold greater luciferase expression compared to non targeted nanorods	[120]

anti-Survivin siRNA. Liposomes were fabricated using gadolinium and rhodamine conjugated lipids as starting materials thus conferring dual MRI and FI capabilities. The nanocarrier manifested superior MRI contrast as compared to clinical agent Dotarem® and enhanced tumour suppression as compared to controls.¹²² Medarova et al. fabricated SPIONs conjugated with Cy5.5 and loaded with siRNA targeting enhanced GFP for MRI, optical imaging, and gene delivery. The nanoprobe demonstrated good gene silencing efficacy in mouse pancreatic islets expressing enhanced GFP thus underscoring the potential of the nanoprobe in image-guided gene delivery to pancreatic islets for treatment of diabetes.¹²³ Bryson et al. fabricated polymer beacons comprising systematically repeated Gd³⁺ chelates or Eu³⁺ chelates within an oligoethyleneamine framework. These beacons were used to bind fluorescently labelled pDNA. The nanocarriers were found to be non-toxic, protected DNA from nuclease degradation and showed successful gene delivery to HeLa cells. The nanocarriers permit imaging at both nm/μm scale (Eu³⁺ by luminescence microscopy) and at sub-mm scale (Gd³⁺ by MRI).¹²⁴ Silica nanotubes are excellent scaffolds for fabrication of multifunctional nanoprobe because of the inner void and outer space which can be utilized to incorporate various imaging and therapeutic components. Namgung et al. loaded IONPs and green fluorescent QDs within the inner voids whereas the outer surface was conjugated with low molecular weight branched PEI (BPEI) to bind pDNA. The nanovector showed 500 fold higher transfection efficacy as compared to branched PEI.¹²⁵

4.2.4. Targeted Theranostic Nanocarriers Providing Dual Modal Imaging and Gene Delivery

Kumar et al. developed dextran coated magnetic nanoparticles conjugated with peptide (binding to tumour specific antigen α MUC-1 which is encountered in 90% of human breast cancer), anti-survivin siRNA and Cy5.5

for targeted dual MR/optical imaging and gene delivery. Intravenous injection of the nanovector once a week over 2 weeks showed significant levels of necrosis and apoptosis in the tumors.¹²⁶ Lee et al. synthesized manganese doped IONPs followed by functionalization with RGD peptide and siRNA-Cy5 dye conjugate via disulfide linkages. The nanoparticles were internalized more efficiently into $\alpha_v\beta_3$ integrin expressing cells as compared to $\alpha_v\beta_3$ non-expressing cells and demonstrated good gene silencing activity.¹²⁷

4.3. Simultaneous Imaging and Photothermal Therapy

Photothermal therapy (PTT) involves conversion of light energy into heat which destroys target tissue by denaturation of cellular proteins.¹²⁸ PTT has recently become very popular for cancer treatment owing to its non-invasiveness compared to surgery and chemotherapy. Initially laser excitation of only a few dyes which had the capacity to absorb light was used in PTT. But today, gold nanostructures in the form of nanoshells, nanorods, nanocages have surfaced as potential candidates for PTT.¹²⁹ Spherical gold nanoparticles absorb light of wavelength 500–600 nm, which is not suitable for PTT. Variation of the configuration to nanocage, nanorod or nanoshell results in absorption in the NIR range which is desirable as soft tissue, water and haemoglobin absorb weakly in this range.³ Gold nanoparticles exhibit several positive attributes for PTT such as biocompatibility, tunable optical characteristics, and ability to be surface modified using specific ligands. The absorption efficacy of gold nanostructures is 4 to 5 times greater than photothermal dyes and also they do not undergo photo bleaching.¹³⁰ However, a major downside associated with most agents used for PTT is the destruction of normal tissue along with tumour tissue. Magnetic nanoparticles are able to surmount this problem because they can be accumulated exclusively

in the tumour by application of magnetic field. Hence, if nanoparticles for PTT are also endowed with imaging capacity, it is possible to distinguish between normal and tumour affected regions prior to PTT and selectively subject the target area to PTT, thereby sparing the normal tissues from photothermal ablation.

Researchers have created numerous theranostic nanocarriers capable of simultaneous imaging and PTT. They can be classified into four categories:

4.3.1. Theranostic Nanocarriers Providing Unimodal Imaging and Photothermal Therapy

Ji et al. prepared nanoparticles in which silica coated IONPs (66 nm) formed the core surrounded by a gold nanoshell (8 nm thickness). Functionalization of the silica surface with amine groups facilitated the attachment of negatively charged gold nanoseeds onto it. The nanoshells were then PEGylated. The nanoshells performed dual functions of MRI and magnetic field guided PTT.¹³¹ Ke et al. developed a bifunctional nanoprobe for simultaneous US imaging and PTT. They loaded gold nanorods (GNR) in poly(lactic acid) microcapsules and demonstrated photothermal killing of HeLa cells after incubation with nanoprobe for 1 hour followed by exposure to NIR light for 10 minutes. US imaging was also demonstrated *in vivo* using New Zealand white rabbits.¹³² Zhou et al. developed CuS nanoparticles (11 nm) for simultaneous PET imaging and PTT. The nanoparticles showed excellent absorption in the NIR region and exhibited photothermal effect against U87 tumour cells both *in vitro* and *in vivo*.¹³³

4.3.2. Targeted Theranostic Nanocarriers Providing Unimodal Imaging and Photothermal Therapy

Day et al. developed HER-2 antibody conjugated gold–gold sulphide nanoparticles for targeted NIR imaging using two photon microscopy and PTT. Two-photon microscopy delivers two NIR photons simultaneously to the gold nanoparticles thus producing images of better resolution. Gold–gold sulphide nanoparticles are smaller in size (25–50 nm) as compared to gold nanoshells (120–150 nm), possess superior light absorbing capacity than gold nanoshells, desirable distribution pattern with greater uptake and retention in tumour regions relative to RES organs, do not require capping with surfactants and, their surface is amenable to functionalization with biomolecules for targeting purpose. The study demonstrated targeted imaging of SK-BR-3 breast carcinoma cells using laser power of 1 mW and targeted photothermal destruction using laser power of 50 mW. The nanoparticles demonstrated the potential to image cancer cells before initiating PTT, thus eliminating unnecessary damage to surrounding normal tissues.¹³⁴

Matrix metalloproteinases are zinc-dependent proteins that play a crucial role in cancer metabolism. Yi et al. developed matrix metalloproteinase (MMP) sensitive GNR by conjugating a peptide sequence (Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys) which is degraded by MMP to a NIR fluorescent dye Cy5.5 and then functionalized the surface of GNR with the peptide sequence-dye conjugate. The fluorescence of Cy5.5 was quenched at the surface of GNR, and after exposure to MMP, the peptide sequence was degraded which resulted in recovery of fluorescence of Cy5.5. The nanorods demonstrated NIR imaging as well as targeted PTT both *in vitro* using HeLa cells and *in vivo* in mouse tumour xenografts.¹³⁵ Toxicity of cetyltrimethylammoniumbromide (CTAB) on the surface of GNR poses a hurdle to their application. Hence, to overcome this problem, Li et al. applied PAMAM dendrimer onto the surface of nanorods to replace CTAB molecules. cRGD was also attached to dendrimer modified nanorods to achieve targeted PTT. The nanoprobe increased the survival of mice considerably as compared to controls.¹³⁶ Huang et al. developed silica modified GNR conjugated with folic acid. Silica modification replaced CTAB on the surface of GNR enhancing their biocompatibility. The nanocarriers showed specific targeting, improved radiation therapy and PTT against gastric cancer MGC803 cells *in vitro* and also demonstrated imaging via CT *in vivo*.¹³⁷

Table VI lists some more theranostic nanocarriers that have been utilized for monitoring targeted PTT.

4.3.3. Theranostic Nanocarriers Providing Dual Modal Imaging and Photothermal Therapy

Dong et al. developed nanocomposites comprising a core of superparamagnetic Fe₃O₄ nanoparticles and a shell of gold nanoparticles. The nanocomposites provide MRI as well as optical imaging and demonstrated cytotoxicity against MCF-7 cells when exposed to laser of 808 nm. *In vivo* toxicity studies demonstrated safety of the nanocomposites and *in vivo* studies in mice showed an increase in temperature of the tumour from 35 to 60 °C when exposed to NIR laser.¹⁴³

4.3.4. Targeted Theranostic Nanocarriers Providing Dual Modal Imaging and Photothermal Therapy

Chen et al. developed nanocomplexes comprising a gold nanoshell encapsulated in a silica shell, which is doped with SPIO and a NIR dye (ICG). The nanocomplexes were further conjugated with anti-HER2 antibody thus providing dual imaging through MRI and optical imaging and targeted PTT for HER2 over expressing ovarian cancer cells.¹⁴⁴ Bardhan et al. coated gold nanoshells with Fe₃O₄ followed by coating with a layer of silica doped with ICG and functionalization with anti-HER2 antibody. The multifunctional nanoprobe was found to target HER2 positive cells and kill them on exposure to NIR.¹⁴⁵ Wang et al.

Table VI. List of targeted theranostic nanocarriers explored for unimodal imaging and PTT.

Nanocarrier	Imaging	PTT component	Targeting moiety	Key features	Ref.
Magnetic-particle gold nanocomposites	MRI	Gold	Erbix	Synergistic activity of Erbix and antibody	[138]
Hybrid nanoparticles of gold and iron oxide	MRI	Gold	scFv against A33 antigen on colorectal cancer cells	99% thermal ablation of A33 positive cells on exposure to laser treatment for six minutes at 31.5 W/cm ²	[139]
Gold nanoparticles	FITC	Gold	Transferrin	Reduction of laser power from 1600 W/cm ² to 7 W/cm ² due to targeting	[140]
Gold nanocages	Gold	Gold	HER2 antibody	Targeted PTT of SK-BR-3 cells at threshold cell damage power density of 1.5 W/cm ² (significantly lower than that of GNR (10 W/cm ²) and gold nanoshells (35 W/cm ²))	[128]
GNR	Gold	Gold	EGFR antibody	Malignant cells required half the laser energy for photothermal ablation compared to the non-malignant ones	[141]
GNR	Gold	Gold	Deltorpin targeting delta opioid receptor	Targeted imaging and photothermal ablation of carcinoma cells	[142]

developed multifunctional nanoprobe for dual FI and MRI and targeted photothermal ablation of human breast cancer cells. They conjugated carboxylic group bearing Fe₃O₄ nanoparticles with PEGylated amine functionalized GNR and conjugated the resulting nanoprobe with anti-HER2 antibody (Herceptin). The gold-iron oxide nanocomposites exhibited superior MRI contrast as compared to free Fe₃O₄ nanoparticles. Targeted PTT was demonstrated using HER2 positive SK-BR-3 cells which showed greater uptake and cell killing as compared to HER2 negative MCF-7 cells.¹⁴⁶ Melancon et al. coated SPIONs with gold nanoshell followed by conjugation with C225 monoclonal antibody against EGFR. The study demonstrated targeted imaging and photothermal ablation both *in vitro* and *in vivo*.¹⁴⁷

4.3.5. Targeted Theranostic Nanocarriers Providing Unimodal Imaging, Drug Delivery and PTT

Few studies have been undertaken to fabricate nanocarriers for PTT as well as chemotherapeutic drug delivery. The idea is to attack the tumour on two independent fronts simultaneously for enhanced therapeutic outcome. Guo et al. fabricated chitosan-GNR hybrid nanospheres, attached FITC to the nanospheres and also loaded cisplatin onto the spherical chitosan matrix. The nanocarriers showed good stability and biocompatibility, and could achieve fluorescent cell imaging, chemotherapeutic and NIR induced destruction of cells within one system.¹⁴⁸ Wang et al. developed PEGylated GNR functionalized with PAA for Dox attachment. The nanoprobe demonstrated release of Dox in acidic environment of pH 5.1 as compared to pH of 7.4.¹⁴⁹ Wu et al. developed a multifunctional nanoplatform for temperature induced temozolide delivery, NIR FI and PTT. They coated Ag-Au nanoparticles with a thermoresponsive non-linear PEG

based hydrogel shell functionalized with hyaluronic acid as targeting moiety. Hyaluronic acid binds to CD44 receptors present on mouse melanoma B16F10 cells and hence the internalization of nanocarriers (less than 100 nm) was enhanced as compared to non-targeted nanocarriers. The Ag-Au core provided NIR FI capability. Thermal exposure (endogenous or exogenous heating) of the system results in shrinkage of the nanogel permitting the expulsion of the drug molecules. The therapeutic efficacy of hybrid nanogels was higher for chemothermal therapy as compared to PTT alone and chemotherapy alone thus establishing the synergistic effect of chemotherapy and PTT.¹⁵⁰

4.4. Combined Imaging and Photodynamic Therapy

Photodynamic therapy (PDT) is a form of cancer therapy in which light sensitive molecules called photosensitizers (PS) are delivered to the area of interest with subsequent irradiation with light of appropriate wavelength. PS on irradiation are activated and release energy into the environment in the form of reactive oxygen species (ROS) such as singlet oxygen ¹O₂. These ROS cause irreversible harm to the diseased tissues.¹⁵¹ PS accepted for clinical use include 5-aminolevulinic acid (ALA) as Levulan®, methyl ester of ALA (Metvix®), and meso-tetrahydroxyphenyl-chlorin (Foscan®).¹⁵² An important benefit of PDT is that it can be used repetitively without any immunosuppressive or myelosuppressive results. It can also be used after surgery, radiotherapy, and chemotherapy.¹⁵³ One major issue that needs to be tackled in PDT is the specific delivery of PS. Selective accumulation of the PS in the target area with minimal or no uptake in the non-target area is desired.¹⁵² Hence, if nanoparticles for PDT are also endowed with imaging capacity, it is possible to differentiate between normal and cancerous tissue prior to initiation

of therapy and thereby spare the normal tissues from destruction.

Theranostic nanocarriers for PDT can be divided into three categories:

4.4.1. *Theranostic Nanocarriers Providing Unimodal Imaging and Photodynamic Therapy*

Cinteza et al. prepared polymeric micelles of diacylphospholipid-PEG loaded with the PS 2-[1-hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH) and magnetic Fe₃O₄ nanoparticles for magnetically controlled PDT. Magnetic field guidance resulted in much greater cellular uptake as compared to the cell cultures not exposed to magnetic field.¹⁵⁴ Chen et al. developed silica nanoparticles (20–30 nm) containing Fe₃O₄ and 2,7,12,18-Tetramethyl-3,8-di-(1-propoxyethyl)-13, 17-bis-(3-hydroxypropyl) porphyrin (PHPP). The nanocarriers were biocompatible, and demonstrated significant PDT efficacy on irradiation. Inclusion of magnetic component endows the system with capacity to perform MRI, targeted PDT as well as magnetic hyperthermia.¹⁵⁵ He et al. developed a theranostic nanoplateform of size 105 nm for simultaneous optical imaging and PDT by encapsulating methylene blue in phosphonate terminated silica matrix. The nanocarriers protected methylene blue against enzymatic degradation and leakage and demonstrated good photodynamic activity against both HeLa cells *in vitro* and xenograft tumour *in vivo*.¹⁵¹ Zhang et al. designed theranostic nanocomposites comprising a dye (FITC) containing non-porous silica core surrounded by a mesoporous silica shell to which PS (hematoporphyrin) was covalently attached. The photo-oxidation efficiency of nanocomposites was found to be superior as compared to that of a homogenous solution. Also, the nonporous silica core protected dye from photobleaching and damage due to singlet oxygen; and the mesoporous shell permitted the entry of oxygen to react with PS to produce ROS.¹⁵⁶ Poon et al. developed an agent for PDT comprising an amphiphilic ruthenium polypyridyl appended porphyrin. The agent demonstrated two photon induced fluorescence, two-photon induced cytotoxic activity, high singlet oxygen yield, and quick and high cellular uptake in nasopharyngeal carcinoma cells. Two-photon excitation of a PS permits more penetration depth through tissues.¹⁵⁷ Zhao et al. developed nanocarriers comprising a core made of GNR and a porphyrin loaded mesoporous silica shell. GNR provided two photon luminescence imaging. Two photon imaging provides greater light penetration depth, low background fluorescence, and 3D imaging ability. The nanocarriers eased the generation and release of singlet oxygen from the matrix and resulted in increased singlet oxygen production efficiency as compared to an aqueous solution of PS.¹⁵⁸ Cheng et al. have designed theranostic mesoporous silica nanoparticles (MSN) conjugated to

Pd-porphyrin for simultaneous phosphorescence imaging and PDT. Hexagonal pores (70–100 nm diameter) are present in the MSN. The huge surface area (approximately 1000 m²/gm) increases the number of PS molecules that can be loaded onto a single particle thus reducing the total photoirradiation energy requirement to achieve desired cytotoxicity. MSN also protect the PS from degradation and release into the systemic circulation. MSN are avidly taken up by cells increasing the intracellular concentration of PS thereby augmenting PDT efficacy. The study demonstrated photodynamic activity of the nanoparticles in MDA-MB-231 breast cancer cells.¹⁵⁹

4.4.2. *Targeted Theranostic Nanocarriers Providing Unimodal Imaging and Photodynamic Therapy*

Reddy et al. developed PEGylated polymeric nanoparticles (40 nm) encapsulating Photofrin and IO. Nanoparticles were labelled with Alexa Flour 750 and conjugated with tumour vasculature targeting F3 peptide. The nanoparticles provided good MRI contrast in glioma bearing rats and good survival rate compared to animals receiving PDT after administration of non targeted nanoparticles or only Photofrin.¹⁶⁰ Leukemia stem cells (LSC) cause cancer development, relapse and drug resistance. Hence, Barth et al. designed CD 117 receptor targeted calcium phosphosilicate nanoparticles loaded with ICG for selective PDT of LSC. An advantage of calcium phosphosilicate nanoparticles is their biocompatibility, and stability at physiological pH. The study demonstrated good PDT efficacy in a murine leukemia model resulting in 29% disease free survival.¹⁶¹ Cheng et al. developed MSN loaded with a NIRF contrast agent ATTO647N in the silica framework, Pd-porphyrin was loaded in the nanopores, and the outer surface was modified with cRGDyK peptide. *In vitro* testing showed good targeting and potent efficacy of the theranostic nanocarrier.¹⁶²

4.4.3. *Theranostic Carriers Providing Stimuli Mediated Release of Photosensitizer*

Koo et al. developed pH responsive block copolymer micelles using MPEG and poly(β -amino ester) containing protoporphyrin IX. Poly(β -amino ester) is pH responsive owing to its tertiary amines. At tumour pH of 6.8, demicellization of these carriers released protoporphyrin IX for FI and PDT. The micelles showed FI and complete destruction of tumours *in vivo*.¹⁶³

4.4.4. *Theranostic Nanocarriers Providing Dual Modal Imaging and Photodynamic Therapy*

Lai et al. developed multifunctional nanoparticles comprising a Fe₃O₄ core within a SiO₂ shell functionalized with iridium complex for magnetic imaging, phosphorescence imaging and PDT. Researchers demonstrated

MRI contrast and photodynamic efficacy of nanoparticles using HeLa cell line.¹⁶⁴ In another study by Huang et al. the PS (Chlorin e6) which could not only generate singlet oxygen on irradiation but also emit fluorescence at longer wavelengths of 660–700 nm suitable for optical imaging was covalently tethered onto magnetic nanoparticles. The developed nanoparticles demonstrated excellent MRI, FI capabilities *in vivo* and also magnetically targeted nanoparticles exhibited superior photodynamic efficacy when compared to non targeted PDT.¹⁶⁵ Schadt et al. prepared superparamagnetic manganese oxide nanoparticles containing protoporphyrin IX. These nanocarriers on exposure to UV light caused cell apoptosis. The nanocarriers could be imaged by MRI as well as by optical imaging since protoporphyrin IX fluoresces.¹⁶⁶

5. THERANOSTIC AGENTS FOR ATHEROSCLEROTIC LESIONS

Cardiovascular diseases remain a major cause of mortality worldwide. Atherosclerosis is the most common cause of cardiovascular diseases. Agents that are used to treat cardiovascular diseases include anticoagulants, thrombolytic agents, antiproliferative agents to prevent restenosis, and PS for macrophage destruction. Atherosclerotic lesions offer a surplus of possible targets such as specific inflammatory cell types, and the upregulation of a number of cell surface receptors such as $\alpha_v\beta_3$ -integrin and vascular cell adhesion molecule-1 (VCAM-1). Macrophages play a pivotal role in formation and development of atherosclerotic plaques. They release inflammatory cytokines and proteases which support the process of inflammation and debase the fibrous cap thus rendering the plaque vulnerable to rupture. Thus, macrophages present a promising target for imaging and therapy of atherosclerosis. Targeted destruction of macrophages has the potential to stabilize vulnerable atherosclerotic lesions.¹⁶⁷ Majority of the research efforts in the field of cardiovascular diseases have focused on either diagnosis or treatment with hardly a few aiming at theranostic nanomedicine. Theranostic nanocarriers definitely have the potential to improve therapeutic efficacy of agents used in atherosclerosis by monitoring their localization and release. Few researchers have nevertheless developed theranostic nanocarriers for atherosclerosis.

Lanza et al. developed paramagnetic nanoparticles containing Dox or PTX for vascular smooth muscle cell targeted MRI coupled with delivery of antiproliferative agents to prevent restenosis after angioplasty. They introduced biotinylated phosphatidylethanolamine in the outer lipid layer of perfluorocarbon microemulsion. Gadolinium contrast moiety was also included in the lipid-surfactant co-mixture. The nanoparticles were targeted with polyclonal antibody against extracellular epitope of recombinant porcine tissue factor. The nanoparticles exhibited

better anti-proliferative activity in vascular smooth muscle cells in culture over a period of three days after a 30 minute exposure as compared to free Dox or PTX.¹⁶⁸ Interleukin-10 (IL-10) is a cytokine having atheroprotective activity. Sun et al. utilized MRI to track IL-10 gene therapy for atherosclerosis. Bone marrow cells were transduced with IL-10/lentivirus and labelled with Feridex. MRI monitoring revealed successful IL-10 gene therapy in atherosclerotic mice.¹⁶⁹

Hua et al. developed US microbubbles carrying tissue plasminogen activator and a peptide for targeting to thrombus (Arg-Gly-Asp-Ser) by lyophilisation technique. The microbubbles provided good contrast in rabbit liver *in vivo* and, provided thrombolytic effect *in vitro* against human blood clots at lower dosages when used with US radiation.¹⁷⁰ Marsh et al. developed fibrin specific liquid perfluorocarbon nanoparticles containing streptokinase. The nanoparticles bound to the human plasma clot surface *in vitro* and enhanced the acoustic contrast of the clot surface. US visualization revealed that streptokinase loaded fibrin specific nanoparticles produced 90% decrease in clot volume of human plasma clots *in vitro* within 60 minutes of exposure. The targeted nanoparticles exhibited substantially superior fibrinolytic activity as compared to free streptokinase. The nanoparticles by virtue of their size (250 nm) remain restricted to the vasculature and as they are modified with fibrin specific antibodies, they possess the potential to target tissue plasminogen activators to the site of action (thrombus) and reduce their undesirable side effects such as haemorrhage and neurodegenerative problems.¹⁷¹

Ma et al. prepared nanoclusters for simultaneous MRI/NIR imaging and therapy of atherosclerotic lesions by self assembly of gold coated IONPs which were further coated with dextran to target dextran receptors present on macrophages. The study demonstrated aggressive uptake of nanocarriers by macrophages as compared to aortic smooth muscle cells and aortic endothelial cells *in vitro*. Also, *in vivo* studies using rabbit atherosclerosis model revealed uptake of nanoclusters by plaque based macrophages followed by excellent NIR contrast. The small size and dextran coating facilitated rapid uptake by macrophages and single 50 ns laser pulse at 755 nm resulted in efficient photothermal destruction of nanocluster loaded macrophages.¹⁷² McCarthy et al. prepared magnetic nanoparticles containing monocrySTALLINE IO and coated them with dextran. Alexa Fluor 750 and PS 5-(4-carboxyphenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin (TPC) were conjugated to nanoparticles. The nanoparticles thus served three purposes of FI, MRI, and targeted delivery of PS for photodynamic destruction of macrophages.¹⁷³ Lim et al. developed hollow type gold nanoparticles functionalized with dextran for targeted NIR imaging and PTT of macrophages. The threshold for selective photothermal annihilation of cells (3.11 W/cm²) was

found to be significantly lower than that of silica/gold nanoshells (35 W/cm²).¹⁷⁴

6. THERANOSTIC AGENTS FOR INFECTION

Theranostic nanocarriers possess the potential to detect infected regions and therefore allow treatment to be carried out exclusively on them. Also, they can be used to monitor therapeutic efficacy. Theranostic nanocarriers for infections are in the nascent stage and more research needs to be done in this area. Few researchers have paved the way for development of infection theranostics whose work has been cited below.

Wang and Irudayaraj designed multifunctional nanoprobe by decorating GNR at the ends (nanodumbbells) and sides (nanonecklaces) with Fe₃O₄ nanoparticles for simultaneous optical recognition and magnetic separation and photothermal destruction of pathogenic bacteria. The nanorods were subsequently conjugated with pathogen specific antibodies for targeted detection, separation and thermal destruction of *Escherichia coli* and *Salmonella typhimurium*. The nanoprobe interacted with the target pathogen for 30 minutes, the bacterial cells bound to the probe were separated magnetically, and then irradiated with NIR light (785 nm) at 50 mW for 15 min for destruction of bacteria. The nanocarrier-bacteria conjugates were then separated and diluted before culturing for 17 hours at 37 °C for examining viability. The targeted nanoprobe demonstrated superior photothermal destruction of bacteria as compared to the non-targeted nanoprobe. The non-targeted nanoprobe failed to bind to the bacteria in sufficient numbers to cause killing of bacteria.¹⁷⁵ Zharov et al. developed 10, 20, and 40 nm sized gold nanoparticles and conjugated them with *Staphylococcus aureus* anti-protein A antibodies for targeted destruction of *Staphylococcus aureus*. Laser exposure resulted in local heating accompanied by bubble formation causing physical damage to the bacterium. Also, nanocluster formation improved the photothermal killing efficacy. The study also demonstrated real time monitoring of conjugation of nanoparticles to bacterium using photothermal imaging since photothermal contrast was more with the bacterial surface conjugated gold nanoparticles as compared to the bacteria alone.¹⁷⁶ Huang et al. designed theranostic nanoeggs for detection and photothermal ablation of bacteria. They first incorporated IONPs inside a silica shell and then attracted gold nanoseeds onto the magnetic silica nanoeggs via electrostatic interactions. Vancomycin was immobilized onto the surface of IO gold nanoeggs which recognized the bacteria by interacting with the D-Ala-D-Ala moieties of peptide units located on bacterial cell walls. Transmission electron microscopy revealed complete coverage of *Acinetobacter baumannii*, *Escherichia coli* O157:H7, *Staphylococcus pyogenes*, *Staphylococcus saprophyticus*,

pan-drug-resistant *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*, and vancomycin-resistant *Enterococcus faecium* by Vancomycin modified nanoeggs whereas only a few non-modified nanoeggs were found to interact with the bacteria. After interaction with the bacteria, the nanoegg-bacteria conjugates were aggregated under the application of an external magnetic field, the NIR radiation was applied to the magnetic spot and photothermal ablation achieved. Magnetic aggregation enhances the efficacy of photothermal destruction and reduces the time required for photothermal ablation. The study also demonstrated similar magnetic aggregation and photothermal efficacy of nanoeggs in serum by spiking bacteria in serum. This method has the potential to be used in patients suffering from severe bacterial infection wherein the bacteria are antibiotic resistant.¹⁷⁷

7. CONCLUSION

Theranostics is a rapidly growing branch of medicine nourished by the active research and progress done in the diverse fields of material science, imaging agents, formulation development and molecular biology. Theranostic nanocarriers possess the potential to revolutionize the arena of health care by combining three cardinal functions of diagnosis, therapy, and targeting in one platform. Theranostic nanocarriers have been extensively explored in the area of oncology for manifold purposes such as chemotherapeutic drug delivery, gene delivery, photothermal therapy, and photodynamic therapy. Recently, theranostic nanocarriers have also been explored albeit to a less extent in cardiovascular medicine and to a very low extent in treatment of infections. However, there are a few pertinent queries that need to be addressed before theranostic carriers make their way into the clinic. Firstly, the disparity in dose required for imaging and treatment results in delivery of inadequate concentrations of the drug or imaging moiety as the case may be. In either case, there is failure of system either as an imaging agent or as a drug delivery vehicle. This issue can be solved with the use of nanocarriers that serve as both drug carriers and imaging moieties such as GNR or SPIONs. Secondly, theranostic nanocarriers are complex systems housing different types of materials in a single platform, the safety of each of the materials used needs to be established. Also, another major issue that needs to be addressed is the likelihood of interactions of the administered theranostic nanocarriers that might occur *in vivo*. Thirdly, a successful theranostic nanocarrier is the one in which neither of the capabilities of imaging or drug delivery are compromised at the cost of the other. Drug incorporation should in no way attenuate the imaging efficacy of the nanoprobe; and at the same time contrast agent should in no way hinder therapeutic agent release. Lastly, fabrication of nanocarriers endowed

with multiple functions increases the cost of manufacturing, thus rendering the systems useful for a limited patient population.

In conclusion, theranostics is a promising field yet in its embryonic stages with just a matter of time before it moves from bench to bedside.

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References and Notes

1. Y. Yu and D. Sun, Superparamagnetic iron oxide nanoparticle 'theranostics' for multimodality tumour imaging, gene delivery, targeted drug and prodrug delivery. *Expert Rev. Clin. Pharmacol.* 3, 117 (2010).
2. S. M. Janib, A. S. Moses, and J. A. MacKay, Imaging and drug delivery using theranostic nanoparticles. *Adv. Drug Deliver. Rev.* 62, 1052 (2010).
3. J. Xie, S. Lee, and X. Chen, Nanoparticle-based theranostic agents. *Adv. Drug Deliver. Rev.* 62, 1064 (2010).
4. C. Sun, J. S. H. Lee, and M. Zhang, Magnetic nanoparticles in MR imaging and drug delivery. *Adv. Drug Deliver. Rev.* 60, 1252 (2008).
5. J. Rao, A. Dragulescu-Andrasi, and H. Yao, Fluorescence imaging *in vivo*: Recent advances. *Curr. Opin. Biotech.* 18, 17 (2007).
6. D. J. Bharali and S. A. Mousa, Emerging nanomedicines for early cancer detection and improved treatment: Current perspective and future promise. *Pharmacol. Therapeut.* 128, 324 (2010).
7. Y.-P. Ho and K. W. Leong, Quantum dot-based theranostics. *Nanoscale* 2, 60 (2010).
8. H. K. Sajja, M. P. East, H. Mao, Y. A. Wang, S. Nie, and L. Yang, Development of multifunctional nanoparticles for targeted drug delivery and non invasive imaging of therapeutic effect. *Curr. Drug Discov. Technol.* 6, 43 (2009).
9. H. M. E. Azzazy, M. M. H. Mansour, and S. C. Kazmierczak, From diagnostics to therapy: Prospects of quantum dots. *Clin. Biochem.* 40, 917 (2007).
10. P. Zrazhevskiy and X. Gao, Multifunctional quantum dots for personalized medicine. *Nano Today* 4, 414 (2009).
11. E. Boisselier and D. Astruc, Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity. *Chem. Soc. Rev.* 38, 1759 (2009).
12. K. W. Ferrara, M. A. Borden, and H. Zhang, Lipid shelled vehicles: Engineering for ultrasound molecular imaging and drug delivery. *Acc. Chem. Res.* 42, 881 (2009).
13. G. A. Koning and G. C. Krijger, Targeted multifunctional lipid-based nanocarriers for image-guided drug delivery. *Anticancer Agents Med. Chem.* 7, 425 (2007).
14. A. L. Klibanov, Microbubble contrast agents: Targeted ultrasound imaging and ultrasound-assisted drug-delivery applications. *Invest. Radiol.* 41, 354 (2006).
15. S. Podell, C. Burrascano, M. Gaal, B. Golec, J. Maniquis, and P. Mehlhaff, Physical and biochemical stability of optison®, an injectable ultrasound contrast agent. *Biotechnol. Appl. Bioc.* 30, 213 (1999).
16. T. Tani, K. Tanabe, M. Ono, M. Katayama, M. Ibuki, S. Mizoguchi, T. Takagi, A. Yamamuro, K. Tamita, K. Yamabe, T. Yagi, K. Nagai, K. Shiratori, and S. Morioka, Quantitative assessment of harmonic power doppler myocardial perfusion imaging with intravenous Levovist™ in patients with myocardial infarction: Comparison with myocardial viability evaluated by thallium-201 single-photon emission computed tomography and coronary flow reserve. *Eur. J. Echocardiogr.* 3, 287 (2002).
17. G. Loudos, G. C. Kagadis, and D. Psimadas, Current status and future perspectives of *in vivo* small animal imaging using radiolabeled nanoparticles. *Eur. J. Radiol.* 78, 287 (2011).
18. W.-Y. Huang and J. J. Davis, Multimodality and nanoparticles in medical imaging. *Dalton T* 40, 6087 (2011).
19. G. Storm and D. J. A. Crommelin, Liposomes: Quo vadis? *Pharmaceutical Science and Technology Today* 1, 19 (1998).
20. A. Samad, Y. Sultana, and M. Aqil, Liposomal drug delivery systems: An update review. *Current Drug Delivery* 4, 297 (2007).
21. U. Kedar, P. Phutane, S. Shidhaye, and V. Kadam, Advances in polymeric micelles for drug delivery and tumor targeting. *Nanomedicine* 6, 714 (2010).
22. N. Nishiyama and K. Kataoka, Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol. Therapeut.* 112, 630 (2006).
23. S. Svenson, Dendrimers as versatile platform in drug delivery applications. *Eur. J. Pharm. Biopharm.* 71, 445 (2009).
24. J. B. Wolinsky and M. W. Grinstaff, Therapeutic and diagnostic applications of dendrimers for cancer treatment. *Adv. Drug Deliver. Rev.* 60, 1037 (2008).
25. J. Pardeike, A. Hommoss, and R. H. Müller, Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *Int. J. Pharm.* 366, 170 (2009).
26. Z. Liu, S. Tabakman, K. Welsher, and H. Dai, Carbon nanotubes in biology and medicine: *In vitro* and *in vivo* detection, imaging and drug delivery. *Nano Res.* 2, 85 (2009).
27. D. J. Bharali, M. Khalil, M. Gurbuz, T. M. Simone, and S. A. Mousa, Nanoparticles and cancer therapy: A concise review with emphasis on dendrimers. *Int. J. Nanomed.* 4, 1 (2009).
28. F. Alexis, J.-W. Rhee, J. P. Richie, A. F. Radovic-Moreno, R. Langer, and O. C. Farokhzad, New frontiers in nanotechnology for cancer treatment. *Urol. Oncol.-Semin. ORI* 26, 74 (2008).
29. E. Blanco, C. W. Kessinger, B. D. Sumer, and J. Gao, Multifunctional micellar nanomedicine for cancer therapy. *Exp. Biol. Med.* 234, 123 (2009).
30. A. R. Menjoge, R. M. Kannan, and D. A. Tomalia, Dendrimer-based drug and imaging conjugates: Design considerations for nanomedical applications. *Drug Discov. Today* 15, 171 (2010).
31. S.-E. Stiriba, H. Frey, and R. Haag, Dendritic polymers in biomedical applications: From potential to clinical use in diagnostics and therapy. *Angew. Chem. Int. Edit.* 41, 1329 (2002).
32. A. Patidar, D. S. Thakur, P. Kumar, and J. Verma, A review on novel lipid based nanocarriers. *International Journal of Pharmacy and Pharmaceutical Sciences* 2, 30 (2010).
33. Z. Liu, K. Chen, C. Davis, S. Sherlock, Q. Cao, X. Chen, and H. Dai, Drug delivery with carbon nanotubes for *in vivo* cancer treatment. *Cancer Res.* 68, 6652 (2008).
34. S. Dhar, Z. Liu, J. Thomale, H. Dai, and S. J. Lippard, Targeted single wall carbon nanotube mediated Pt(IV) prodrug delivery using folate as a homing device. *J. Am. Chem. Soc.* 130, 11467 (2008).
35. A. A. Bhirde, V. Patel, J. Gavard, G. Zhang, A. A. Sousa, A. Masedunskas, R. D. Leapman, R. Weigert, J. S. Gutkind, and J. F. Rusling, Targeted killing of cancer cells *in vivo* and *in vitro* with EGF-directed carbon nanotube-based drug delivery. *ACS Nano* 3, 307 (2009).
36. Y. Liu, D. C. Wu, W.-D. Zhang, X. Jiang, C.-B. He, T. S. Chung, S. H. Goh, and K. W. Leong, Polyethylenimine-grafted multi-walled carbon nanotubes for secure noncovalent immobilization and efficient delivery of DNA. *Angew. Chem. Int. Edit.* 44, 4782 (2005).
37. L. Gao, L. Nie, T. Wang, Y. Qin, Z. Guo, D. Yang, and X. Yan, Carbon nanotube delivery of the GFP gene into mammalian cells. *Chem. Bio. Chem.* 7, 239 (2006).
38. Z. Zhang, X. Yang, Y. Zhang, B. Zeng, S. Wang, T. Zhu, R. B. S. Roden, Y. Chen, and R. Yang, Delivery of telomerase

reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes suppresses tumor growth. *Clin. Cancer Res.* 12, 4933 (2006).

39. N. W. S. Kam, Z. Liu, and H. Dai, Functionalization of carbon nanotubes via cleavable disulfide bonds for efficient intracellular delivery of siRNA and potent gene silencing. *J. Am. Chem. Soc.* 127, 12492 (2005).
40. X. Wang, L. Yang, Z. Chen, and D. M. Shin, Application of nanotechnology in cancer therapy and imaging. *CA-Cancer J. Clin.* 58, 97 (2008).
41. H.-M. Yang, H. J. Lee, C. W. Park, S. R. Yoon, S. Lim, B. H. Jung, and J.-D. Kim, Endosome-escapable magnetic poly(amino acid) nanoparticles for cancer diagnosis and therapy. *Chem. Commun.* 47, 5322 (2011).
42. E. Cittadino, M. Ferraretto, E. Torres, A. Maiocchi, B. J. Crielaard, T. Lammers, G. Storm, S. Aime, and E. Terreno, MRI evaluation of the antitumor activity of paramagnetic liposomes loaded with prednisolone phosphate. *Eur. J. Pharm. Sci.* 45, 436 (2012).
43. B. Tian, W. T. Al-Jamal, K. T. Al-Jamal, and K. Kostarelos, Doxorubicin-loaded lipid-quantum dot hybrids: Surface topography and release properties. *Int. J. Pharm.* 416, 443 (2011).
44. X. Feng, F. Lv, L. Liu, H. Tang, C. Xing, Q. Yang, and S. Wang, Conjugated polymer nanoparticles for drug delivery and imaging. *Applied Materials and Interfaces* 2, 2429 (2010).
45. D. Ding, K. Li, Z. Zhu, K.-Y. Pu, Y. Hu, X. Jiang, and B. Liu, Conjugated polyelectrolyte-cisplatin complex nanoparticles for simultaneous *in vivo* imaging and drug tracking. *Nanoscale* 3, 1997 (2011).
46. E. C. Wu, J. S. Andrew, L. Cheng, W. R. Freeman, L. Pearson, and M. J. Sailor, Real-time monitoring of sustained drug release using the optical properties of porous silicon photonic crystal particles. *Biomaterials* 32, 1957 (2011).
47. H.-M. Yang, B. C. Oh, J. H. Kim, T. Ahn, H.-S. Nam, C. W. Park, and J.-D. Kim, Multifunctional poly(aspartic acid) nanoparticles containing iron oxide nanocrystals and doxorubicin for simultaneous cancer diagnosis and therapy. *Colloids Surf. A* 391, 208 (2011).
48. T. K. Jain, J. Richey, M. Strand, D. L. Leslie-Pelecky, C. A. Flask, and V. Labhasetwar, Magnetic nanoparticles with dual functional properties: Drug delivery and magnetic resonance imaging. *Biomaterials* 29, 4012 (2008).
49. Y. Chen, H. Chen, D. Zeng, Y. Tian, F. Chen, J. Feng, and J. Shi, Core/shell structured hollow mesoporous nanocapsules: A potential platform for simultaneous cell imaging and anticancer drug delivery. *ACS Nano* 4, 6001 (2010).
50. H. Wu, G. Liu, X. Wang, J. Zhang, Y. Chen, J. Shi, H. Yang, H. Hu, and S. Yang, Solvothermal synthesis of cobalt ferrite nanoparticles loaded on multiwalled carbon nanotubes for magnetic resonance imaging and drug delivery. *Acta Biomater.* 7, 3496 (2011).
51. K. Kim, J. H. Kim, H. Park, Y.-S. Kim, K. Park, H. Nam, S. Lee, J. H. Park, R.-W. Park, I.-S. Kim, K. Choi, S. Y. Kim, K. Park, and I. C. Kwon, Tumor-homing multifunctional nanoparticles for cancer theragnosis: Simultaneous diagnosis, drug delivery, and therapeutic monitoring. *J. Control Release* 146, 219 (2010).
52. Y. Guo, D. Shi, H. Cho, Z. Dong, A. Kulkarni, G. M. Pauletti, W. Wang, J. Lian, W. Liu, L. Ren, Q. Zhang, G. Liu, C. Huth, L. Wang, and R. C. Ewing, *In vivo* imaging and drug storage by quantum-dot-conjugated carbon nanotubes. *Adv. Funct. Mater.* 18, 2489 (2008).
53. W. Di, X. Ren, H. Zhao, N. Shirahata, Y. Sakka, and W. Qin, Single-phased luminescent mesoporous nanoparticles for simultaneous cell imaging and anticancer drug delivery. *Biomaterials* 32, 7226 (2011).
54. L. Zhang, H. Xue, Z. Cao, A. Keefe, J. Wang, and S. Jiang, Multifunctional and degradable zwitterionic nanogels for targeted delivery, enhanced MR imaging, reduction-sensitive drug release, and renal clearance. *Biomaterials* 32, 4604 (2011).
55. M. Guo, C. Que, C. Wang, X. Liu, H. Yan, and K. Liu, Multifunctional superparamagnetic nanocarriers with folate-mediated and pH-responsive targeting properties for anticancer drug delivery. *Biomaterials* 32, 185 (2011).
56. X. Li, Y. Qian, T. Liu, X. Hu, G. Zhang, Y. You, and S. Liu, Amphiphilic multiarm star block copolymer-based multifunctional unimolecular micelles for cancer targeted drug delivery and MR imaging. *Biomaterials* 32, 6595 (2011).
57. V. Bagalkot, L. Zhang, E. Levy-Nissenbaum, S. Jon, P. W. Kantoff, R. Langer, and O. C. Farokhzad, Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on bi-fluorescence resonance energy transfer. *Nano Lett.* 7, 3065 (2007).
58. S. Santra, C. Kaitanis, and J. M. Perez, Cytochrome *c* encapsulating theranostic nanoparticles: A novel bifunctional system for targeted delivery of therapeutic membrane-impermeable proteins to tumors and imaging of cancer therapy. *Mol. Pharm.* 7, 1209 (2010).
59. K. Lee, H. Lee, K. H. Bae, and T. G. Park, Heparin immobilized gold nanoparticles for targeted detection and apoptotic death of metastatic cancer cells. *Biomaterials* 31, 6530 (2010).
60. M. Nurunnabi, K. J. Cho, J. S. Choi, K. M. Huh, and Y.-K. Lee, Targeted near-IR QDs-loaded micelles for cancer therapy and imaging. *Biomaterials* 31, 5436 (2010).
61. M. S. Muthu, S. A. Kulkarni, A. Raju, and S.-S. Feng, Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots. *Biomaterials* 33, 3494 (2012).
62. J. S. Guthi, S.-G. Yang, G. Huang, S. Li, C. Khemtong, C. W. Kessinger, M. Peyton, J. D. Minna, K. C. Brown, and J. Gao, MRI-visible micellar nanomedicine for targeted drug delivery to lung cancer cells. *Mol. Pharm.* 7, 32 (2010).
63. N. Nasongkla, E. Bey, J. Ren, H. Ai, C. Khemtong, J. S. Guthi, S.-F. Chin, A. D. Sherry, D. A. Boothman, and J. Gao, Multifunctional polymeric micelles as cancer-targeted, mri-ultrasensitive drug delivery systems. *Nano Lett.* 6, 2427 (2006).
64. M. K. Yu, J. Park, Y. Y. Jeong, W. K. Moon, and S. Jon, Integrin-targeting thermally cross-linked superparamagnetic iron oxide nanoparticles for combined cancer imaging and drug delivery. *Nanotechnology* 21, 1 (2010).
65. X. Yang, J. J. Grailler, S. Pilla, D. A. Steeber, S. Gong, and X. Shuai, Multifunctional polymeric vesicles for targeted drug delivery and imaging. *Biofabrication* 2, 1 (2010).
66. Y. Ling, K. Wei, Y. Luo, X. Gao, and S. Zhong, Dual docetaxel/superparamagnetic iron oxide loaded nanoparticles for both targeting magnetic resonance imaging and cancer therapy. *Biomaterials* 32, 7139 (2011).
67. H. Wu, G. Liu, S. Zhang, J. Shi, L. Zhang, Y. Chen, F. Chen, and H. Chen, Biocompatibility, MR imaging and targeted drug delivery of a rattle-type magnetic mesoporous silica nanosphere system conjugated with PEG and cancer-cell-specific ligands. *J. Mater. Chem.* 21, 3037 (2011).
68. C. Sun, C. Fang, Z. Stephen, O. Veisich, S. Hansen, D. Lee, R. G. Ellenbogen, J. Olson, and M. Zhang, Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles. *Nanomedicine* 3, 495 (2008).
69. G. Hong, R. Yuan, B. Liang, J. Shen, X. Yang, and X. Shuai, Folate-functionalized polymeric micelle as hepatic carcinoma-targeted, MRI-ultrasensitive delivery system of antitumor drugs. *Biomed. Microdevices* 10, 693 (2008).
70. J. Yang, C.-H. Lee, J. Park, S. Seo, E.-K. Lim, Y. J. Song, J.-S. Suh, H.-G. Yoon, Y.-M. Huh, and S. Haam, Antibody conjugated magnetic PLGA nanoparticles for diagnosis and treatment of breast cancer. *J. Mater. Chem.* 17, 2695 (2007).
71. M. M. Yallapu, S. P. Foy, T. K. Jain, and V. Labhasetwar, PEG-functionalized magnetic nanoparticles for drug delivery and magnetic resonance imaging applications. *Pharm. Res.* 27, 2283 (2010).

72. F. Dilnawaz, A. Singh, C. Mohanty, and S. K. Sahoo, Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. *Biomaterials* 31, 3694 (2010).
73. A. Z. Wang, V. Bagalkot, C. C. Vasilgiou, F. Gu, F. Alexis, L. Zhang, M. Shaikh, K. Yuet, M. J. Cima, R. Langer, P. W. Kantoff, N. H. Bander, S. Jon, and O. C. Farokhzad, Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. *Chem. Med. Chem.* 3, 1311 (2008).
74. C. Grange, S. Geninatti-Crich, G. Esposito, D. Alberti, L. Tei, B. Bussolati, S. Aime, and G. Camussi, Combined delivery and magnetic resonance imaging of neural cell adhesion molecule-targeted doxorubicin-containing liposomes in experimentally induced kaposi's sarcoma. *Cancer Res.* 70, 2180 (2010).
75. M. E. Mathew, J. C. Mohan, K. Manzoor, S. V. Nair, H. Tamura, and R. Jayakumar, Folate conjugated carboxymethyl chitosan-manganese doped zinc sulphide nanoparticles for targeted drug delivery and imaging of cancer cells. *Carbohydr Polym.* 80, 442 (2010).
76. T.-D. Way, C.-J. Chang, and C.-W. Lin, Bioconjugated fluorescent polymeric nanoparticles for imaging and targeted therapy of HER2-overexpressing cancer cells. *J. Fluoresc.* 21, 1669 (2011).
77. H. Zhu, F. Liu, J. Guo, J. Xue, Z. Qian, and Y. Gu, Folate-modified chitosan micelles with enhanced tumor targeting evaluated by near infrared imaging system. *Carbohydr. Polym.* 86, 1118 (2011).
78. H.-C. Tsai, W.-H. Chang, C.-L. Lo, C.-H. Tsai, C.-H. Chang, T.-W. Ou, T.-C. Yen, and G.-H. Hsiue, Graft and diblock copolymer multifunctional micelles for cancer chemotherapy and imaging. *Biomaterials* 31, 2293 (2010).
79. D. Kim, Y. Y. Jeong, and S. Jon, A drug-loaded aptamer-gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. *ACS Nano* 4, 3689 (2010).
80. C. Wang, L. Cheng, and Z. Liu, Drug delivery with upconversion nanoparticles for multi-functional targeted cancer cell imaging and therapy. *Biomaterials* 32, 1110 (2011).
81. J. E. Lee, N. Lee, H. Kim, J. Kim, S. H. Choi, J. H. Kim, T. Kim, I. C. Song, S. P. Park, W. K. Moon, and T. Hyeon, Uniform mesoporous dye-doped silica nanoparticles decorated with multiple magnetite nanocrystals for simultaneous enhanced magnetic resonance imaging, fluorescence imaging, and drug delivery. *J. Am. Chem. Soc.* 132, 552 (2010).
82. J. Choi, K. Kim, T. Kim, G. Liu, A. Bar-Shir, T. Hyeon, M. T. McMahon, J. W. M. Bulte, J. P. Fisher, and A. A. Gilad, Multimodal imaging of sustained drug release from 3-Dpoly(propylene fumarate) (PPF) scaffolds. *J. Control Release* 156, 239 (2011).
83. F. Chen, P. Huang, Y.-J. Zhu, J. Wu, C.-L. Zhang, and D.-X. Cui, The photoluminescence, drug delivery and imaging properties of multifunctional $\text{Eu}^{3+}/\text{Gd}^{3+}$ dual-doped hydroxyapatite nanorods. *Biomaterials* 32, 9031 (2011).
84. J.-H. Park, G. von Maltzahn, E. Ruoslahti, S. N. Bhatia, and M. J. Sailor, Micellar hybrid nanoparticles for simultaneous magnetofluorescent imaging and drug delivery. *Angew. Chem.* 120, 7394 (2008).
85. S. Santra, C. Kaittanis, J. Grimm, and J. M. Perez, Drug/dye-loaded, multifunctional iron oxide nanoparticles for combined targeted cancer therapy and dual optical/magnetic resonance imaging. *Small* 5, 1862 (2009).
86. M. Liong, J. Lu, M. Kovochich, T. Xia, S. G. Ruehm, A. E. Nel, F. Tamanoi, and J. I. Zink, Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *ACS Nano* 2, 889 (2008).
87. H.-S. Cho, Z. Dong, G. M. Pauletti, J. Zhang, H. Xu, H. Gu, L. Wang, R. C. Ewing, C. Huth, F. Wang, and D. Shi, Fluorescent, superparamagnetic nanospheres for drug storage, targeting, and imaging: A multifunctional nanocarrier system for cancer diagnosis and treatment. *ACS Nano* 4, 5398 (2010).
88. M. O. Abdalla, P. Karna, H. K. Sajja, H. Mao, C. Yates, T. Turner, and R. Aneja, Enhanced nescapine delivery using α PAR-targeted optical-MR imaging trackable nanoparticles for prostate cancer therapy. *J. Control Release* 149, 314 (2011).
89. W. Wu, M. Aiello, T. Zhou, A. Berliner, P. Banerjee, and S. Zhou, *In-situ* immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery. *Biomaterials* 31, 3023 (2010).
90. F. M. Kievit, F. Y. Wang, C. Fang, H. Mok, K. Wang, J. R. Silber, R. G. Ellenbogen, and M. Zhang, Doxorubicin loaded iron oxide nanoparticles overcome multidrug resistance in cancer *in vitro*. *J. Control Release* 152, 76 (2011).
91. W. Wu, J. Shen, P. Banerjee, and S. Zhou, Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery. *Biomaterials* 31, 8371 (2010).
92. K. Kono, S. Nakashima, D. Kokuryo, I. Aoki, H. Shimomoto, S. Aoshima, K. Maruyama, E. Yuba, C. Kojima, A. Harada, and Y. Ishizaka, Multi-functional liposomes having temperature-triggered release and magnetic resonance imaging for tumor-specific chemotherapy. *Biomaterials* 32, 1387 (2011).
93. M. de Smet, S. Langereis, S. van den Bosch, and H. Grull, Temperature sensitive liposomes for doxorubicin delivery under MRI guidance. *J. Control Release* 143, 120 (2010).
94. T. Tagami, W. D. Foltz, M. J. Ernsting, C. M. Lee, I. F. Tannock, J. P. May, and S.-D. Li, MRI monitoring of intratumoral drug delivery and prediction of the therapeutic effect with a multifunctional thermosensitive liposome. *Biomaterials* 32, 6570 (2011).
95. C. Sanson, O. Diou, J. Thevenot, E. Ibarboure, A. Soum, A. Brulet, S. Miraux, E. Thiaudiere, S. Tan, A. Brisson, V. Dupuis, O. Sandre, and S. Lecommandoux, Doxorubicin loaded magnetic polymersomes: Theranostic nanocarriers for mr imaging and magneto-chemotherapy. *ACS Nano* 5, 1122 (2011).
96. Z. Gao, A. M. Kennedy, D. A. Christensen, and N. Y. Rapoport, Drug-loaded nano/microbubbles for combining ultrasonography and targeted chemotherapy. *Ultrasonics* 48, 260 (2008).
97. N. Rapoport, Z. Gao, and A. Kennedy, Multifunctional nanoparticles for combining ultrasonic tumor imaging and targeted chemotherapy. *J. Natl. Cancer Inst.* 99, 1095 (2007).
98. N. Kohler, C. Sun, J. Wang, and M. Zhang, Methotrexate-modified superparamagnetic nanoparticles and their intracellular uptake into human cancer cells. *Langmuir* 21, 8858 (2005).
99. R. Savla, O. Taratula, O. Garbuzenko, and T. Minko, Tumor targeted quantum dot-mucin 1 aptamer-doxorubicin conjugate for imaging and treatment of cancer. *J. Control Release* 153, 16 (2011).
100. N. Rapoport, K.-H. Nam, R. Gupta, Z. Gao, P. Mohan, A. Payne, N. Todd, X. Liu, T. Kim, J. Shea, C. Scaife, D. L. Parker, E.-K. Jeong, and A. M. Kennedy, Ultrasound-mediated tumor imaging and nanotherapy using drug loaded, block copolymer stabilized perfluorocarbon nanoemulsions. *J. Control Release* 153, 4 (2011).
101. W. Wu, J. Shen, Z. Gai, K. Hong, P. Banerjee, and S. Zhou, Multi-functional core-shell hybrid nanogels for pH-dependent magnetic manipulation, fluorescent pH-sensing, and drug delivery. *Biomaterials* 32, 9876 (2011).
102. M. Das, D. Mishra, P. Dhak, S. Gupta, T. K. Maiti, A. Basak, and P. Pramanik, Biofunctionalized, phosphonate-grafted, ultra-small iron oxide nanoparticles for combined targeted cancer therapy and multimodal imaging. *Small* 5, 2883 (2009).
103. X. Yang, H. Hong, J. J. Grailer, I. J. Rowland, A. Javadi, S. A. Hurley, Y. Xiao, Y. Yang, Y. Zhang, R. J. Nickles, W. Cai, D. A. Steeber, and S. Gong, cRGD-functionalized, DOX-conjugated, and ^{64}Cu -labeled superparamagnetic iron oxide

nanoparticles for targeted anticancer drug delivery and PET/MR imaging. *Biomaterials* 32, 4151 (2011).

104. P. Zou, Y. Yu, Y. A. Wang, Y. Zhong, A. Welton, C. Galban, S. Wang, and D. Sun, Superparamagnetic iron oxide nanotheranostics for targeted cancer cell imaging and pH-dependent intracellular drug release. *Mol. Pharm.* 7, 1974 (2010).
105. K. H. Bae, K. Lee, C. Kim, and T. G. Park, Surface functionalized hollow manganese oxide nanoparticles for cancer targeted siRNA delivery and magnetic resonance imaging. *Biomaterials* 32, 176 (2011).
106. S.-J. Cheong, C.-M. Lee, S.-L. Kim, H.-J. Jeong, E.-M. Kim, E.-H. Park, D. W. Kim, S. T. Lim, and M.-H. Sohn, Superparamagnetic iron oxide nanoparticles-loaded chitosan-linoleic acid nanoparticles as an effective hepatocyte-targeted gene delivery system. *Int. J. Pharm.* 372, 169 (2009).
107. G. Bhakta, R. K. Sharma, N. Gupta, S. Cool, V. Nurcombe, and A. Maitra, Multifunctional silica nanoparticles with potentials of imaging and gene delivery. *Nanomed. Nanotechnol.* 7, 472 (2011).
108. X. Feng, Y. Tang, X. Duan, L. Liu, and S. Wang, Lipid-modified conjugated polymer nanoparticles for cell imaging and transfection. *J. Mater. Chem.* 20, 1312 (2010).
109. L. Qi and X. Gao, Quantum dot-ampiphil nanocomplex for intracellular delivery and real-time imaging of siRNA. *ACS Nano* 2, 1403 (2008).
110. J.-M. Li, M.-X. Zhao, H. Su, Y.-Y. Wang, C.-P. Tan, L.-N. Ji, and Z.-W. Mao, Multifunctional quantum-dot-based siRNA delivery for HPV18 E6 gene silence and intracellular imaging. *Biomaterials* 32, 7978 (2011).
111. M.-X. Zhao, J.-M. Li, L. Du, C.-P. Tan, Q. Xia, Z.-W. Mao, and L.-N. Ji, Targeted cellular uptake and siRNA silencing by quantum-Dot nanoparticles coated with β -cyclodextrin coupled to amino acids. *Chem. Eur. J.* 17, 5171 (2011).
112. M. S. Shim, C. S. Kim, Y.-C. Ahn, Z. Chen, and Y. J. Kwon, Combined multimodal optical imaging and targeted gene silencing using stimuli-transforming nanotheranostics. *J. Am. Chem. Soc.* 132, 8316 (2010).
113. C. Guihua, C. Wenjie, W. Zhuang, Y. Renxu, L. Hua, G. Jinming, and S. Xintao, MRI-visible polymeric vector bearing CD3 single chain antibody for gene delivery to T cells for immunosuppression. *Biomaterials* 30, 1962 (2009).
114. S. Jiang, Y. Zhang, K. M. Lim, E. K. W. Sim, and L. Ye, NIR-to-visible upconversion nanoparticles for fluorescent labeling and targeted delivery of siRNA. *Nanotechnology* 20, 1 (2009).
115. D. W. Bartlett, H. Su, I. J. Hildebrandt, W. A. Weber, and M. E. Davis, Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticles measured by multimodality *in vivo* imaging. *Proc. Natl. Acad. Sci. USA* 104, 15549 (2007).
116. O. Veiseh, F. M. Kievit, C. Fang, N. Mu, S. Jana, M. C. Leung, H. Mok, R. G. Ellenbogen, J. O. Park, and M. Zhang, Chlorotoxin bound magnetic nanovector tailored for cancer cell targeting, imaging, and siRNA delivery. *Biomaterials* 31, 8032 (2010).
117. F. M. Kievit, O. Veiseh, C. Fang, N. Bhattarai, D. Lee, R. G. Ellenbogen, and M. Zhang, Chlorotoxin labeled magnetic nanovectors for targeted gene delivery to glioma. *ACS Nano* 4, 4587 (2010).
118. A. M. Derfus, A. A. Chen, D.-H. Min, E. Ruoslahti, and S. N. Bhatia, Targeted quantum dot conjugates for siRNA delivery. *Bioconjug. Chem.* 18, 1391 (2007).
119. Q. Yuan, E. Lee, W. A. Yeudall, and H. Yang, Dendrimer-triglycine-EGF nanoparticles for tumor imaging and targeted nucleic acid and drug delivery. *Oral Oncol.* 46, 698 (2010).
120. A. K. Salem, P. C. Searson, and K. W. Leong, Multifunctional nanorods for gene delivery. *Nat. Mater.* 2, 668 (2003).
121. Z. Medarova, M. Kumar, S. W. Ng, and A. Moore, Development and application of a dual-purpose nanoparticle platform for delivery and imaging of siRNA in tumors. *Methods Mol. Biol.* 555, 1 (2009).
122. G. D. Kenny, N. Kamaly, T. L. Kalber, L. P. Brody, M. Sahuri, E. Shamsaei, A. D. Miller, and J. D. Bell, Novel multifunctional nanoparticle mediates siRNA tumour delivery, visualisation and therapeutic tumour reduction *in vivo*. *J. Control Release* 149, 111 (2011).
123. Z. Medarova, M. Kumar, S. Ng, J. Yang, N. Barteneva, N. V. Evgenov, V. Petkova, and A. Moore, Multifunctional magnetic nanocarriers for image-tagged siRNA delivery to intact pancreatic islets. *Transplantation* 86, 1170 (2008).
124. J. M. Bryson, K. M. Fichter, W.-J. Chu, J.-H. Lee, J. Li, L. A. Madsen, P. M. McLendon, and T. M. Reineke, Polymer beads for luminescence and magnetic resonance imaging of DNA delivery. *Proc. Natl. Acad. Sci. USA* 106, 16913 (2009).
125. R. Namgung, Y. Zhang, Q. L. Fang, K. Singha, H. J. Lee, I. K. Kwon, Y. Y. Jeong, I.-K. Park, S. J. Son, and W. J. Kim, Multifunctional silica nanotubes for dual-modality gene delivery and MR imaging. *Biomaterials* 32, 3042 (2011).
126. M. Kumar, M. Yigit, G. Dai, A. Moore, and Z. Medarova, Image-guided breast tumor therapy using a small interfering RNA nano-drug. *Cancer Res.* 70, 7553 (2010).
127. J.-H. Lee, K. Lee, S. H. Moon, Y. Lee, T. G. Park, and J. Cheon, All-in-one target-cell-specific magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery. *Angew. Chem.* 121, 4238 (2009).
128. J. Chen, D. Wang, J. Xi, L. Au, A. Siekkinen, A. Warsen, Z.-Y. Li, H. Zhang, Y. Xia, and X. Li, Immuno gold nanocages with tailored optical properties for targeted photothermal destruction of cancer cells. *Nano Lett.* 7, 1318 (2007).
129. M. E. Gindy and R. K. Prud'homme, Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. *Expert Opin. Drug Del.* 6, 865 (2009).
130. K. Park, S. Lee, E. Kang, K. Kim, K. Choi, and I. C. Kwon, New generation of multifunctional nanoparticles for cancer imaging and therapy. *Adv. Funct. Mater.* 19, 1553 (2009).
131. X. Ji, R. Shao, A. M. Elliott, R. J. Stafford, E. Esparza-Coss, J. A. Bankson, G. Liang, Z.-P. Luo, K. Park, J. T. Markert, and C. Li, Bifunctional gold nanoshells with a superparamagnetic iron oxide-silica core suitable for both MR imaging and photothermal therapy. *J. Phys. Chem. C* 111, 6245 (2007).
132. H. Ke, J. Wang, Z. Dai, Y. Jin, E. Qu, Z. Xing, C. Guo, J. Liu, and X. Yue, Bifunctional gold nanorod-loaded polymeric microcapsules for both contrast-enhanced ultrasound imaging and photothermal therapy. *J. Mater. Chem.* 21, 5561 (2011).
133. M. Zhou, R. Zhang, M. Huang, W. Lu, S. Song, M. P. Melancon, M. Tian, D. Liang, and C. Li, A chelator-free multifunctional [^{64}Cu]CuS nanoparticle platform for simultaneous micro-PET/CT imaging and photothermal ablation therapy. *J. Am. Chem. Soc.* 132, 15351 (2010).
134. E. S. Day, L. R. Bickford, J. H. Slater, N. S. Riggall, R. A. Drezek, and J. L. West, Antibody-conjugated gold-gold sulphide nanoparticles as multifunctional agents for imaging and therapy of breast cancer. *Int. J. Nanomed.* 5, 445 (2010).
135. D. K. Yi, I.-C. Sun, J. H. Ryu, H. Koo, C. W. Park, I.-C. Youn, K. Choi, I. C. Kwon, K. Kim, and C.-H. Ahn, Matrix metalloproteinase sensitive gold nanorod for simultaneous bioimaging and photothermal therapy of cancer. *Bioconjug. Chem.* 21, 2173 (2010).
136. Z. Li, P. Huang, X. Zhang, J. Lin, S. Yang, B. Liu, F. Gao, P. Xi, Q. Ren, and D. Cui, RGD-conjugated dendrimer-modified gold nanorods for *in vivo* tumor targeting and photothermal therapy. *Mol. Pharm.* 7, 94 (2010).
137. P. Huang, L. Bao, C. Zhang, J. Lin, T. Luo, D. Yang, M. He, Z. Li, G. Gao, B. Gao, S. Fu, and D. Cui, Folic acid-conjugated silica-modified gold nanorods for X-ray/CT imaging-guided dual-mode radiation and photo-thermal therapy. *Biomaterials* 32, 9796 (2011).
138. J. Lee, J. Yang, H. Ko, S. J. Oh, J. Kang, J.-H. Son, K. Lee, S.-W. Lee, H.-G. Yoon, J.-S. Suh, Y.-M. Huh, and S. Haam,

- Multifunctional magnetic gold nanocomposites: Human epithelial cancer detection via magnetic resonance imaging and localized synchronous therapy. *Adv. Funct. Mater.* 18, 258 (2008).
139. D. K. Kirui, D. A. Rey, and C. A. Batt, Gold hybrid nanoparticles for targeted phototherapy and cancer imaging. *Nanotechnology* 21, 1 (2010).
 140. J.-L. Li, L. Wang, X.-Y. Liu, Z.-P. Zhang, H.-C. Guo, W.-M. Liu, and S.-H. Tang, *In vitro* cancer cell imaging and therapy using transferrin-conjugated gold nanoparticles. *Cancer Lett.* 274, 319 (2009).
 141. X. Huang, I. H. El-Sayed, W. Qian, and M. A. El-Sayed, Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* 128, 2115 (2006).
 142. K. C. Black, N. D. Kirkpatrick, T. S. Troutman, L. Xu, J. Vaguer, R. J. Gillies, J. K. Barton, U. Utzinger, and M. Romanowski, Gold nanorods targeted to delta opioid receptor: Plasmon-resonant contrast and photothermal agents. *Mol. Imaging.* 7, 50 (2008).
 143. W. Dong, Y. Li, D. Niu, Z. Ma, J. Gu, Y. Chen, W. Zhao, X. Liu, C. Liu, and J. Shi, Facile synthesis of monodisperse superparamagnetic Fe₃O₄ Core@hybrid@Au shell nanocomposite for bimodal imaging and photothermal therapy. *Adv. Funct. Mater.* 23, 5392 (2011).
 144. W. Chen, R. Bardhan, M. Bartels, C. Perez-Torres, R. G. Pautler, N. J. Halas, and A. Joshi, A molecularly targeted theranostic probe for ovarian cancer. *Mol. Cancer Ther.* 9, 1028 (2010).
 145. R. Bardhan, W. Chen, C. Perez-Torres, M. Bartels, R. M. Hushka, L. L. Zhao, E. Morosan, R. G. Pautler, A. Joshi, and N. J. Halas, Nanoshells with targeted simultaneous enhancement of magnetic and optical imaging and photothermal therapeutic response. *Adv. Funct. Mater.* 19, 3901 (2009).
 146. C. Wang, J. Chen, T. Talavage, and J. Irudayaraj, Gold nanorod/Fe₃O₄ nanoparticle "nano-pearl-necklaces" for simultaneous targeting, dual-mode imaging, and photothermal ablation of cancer cells. *Angew. Chem. Int. Edit.* 48, 2759 (2009).
 147. M. P. Melancon, W. Lu, M. Zhong, M. Zhou, G. Liang, A. M. Elliott, J. D. Hazle, J. N. Myers, C. Li, and R. J. Stafford, Targeted multifunctional gold-based nanoshells for magnetic resonance-guided laser ablation of head and neck cancer. *Biomaterials* 32, 7600 (2011).
 148. R. Guo, L. Zhang, H. Qian, R. Li, X. Jiang, and B. Liu, Multifunctional nanocarriers for cell imaging, drug delivery, and near-IR photothermal therapy. *Langmuir* 26, 5428 (2010).
 149. T. Wang, X. Zhang, Y. Pan, X. Miao, Z. Su, C. Wang, and X. Li, Fabrication of doxorubicin functionalized gold nanorod probes for combined cancer imaging and drug delivery. *Dalton T* 40, 9789 (2011).
 150. W. Wu, J. Shen, P. Banerjee, and S. Zhou, Core-shell hybrid nanogels for integration of optical temperature-sensing, targeted tumor cell imaging, and combined chemo-photothermal treatment. *Biomaterials* 31, 7555 (2010).
 151. X. He, X. Wu, K. Wang, B. Shi, and L. Hai, Methylene blue-encapsulated phosphonate-terminated silica nanoparticles for simultaneous *in vivo* imaging and photodynamic therapy. *Biomaterials* 30, 5601 (2009).
 152. D. K. Chatterjee, L. S. Fong, and Y. Zhang, Nanoparticles in photodynamic therapy: An emerging paradigm. *Adv. Drug Deliver. Rev.* 60, 1627 (2008).
 153. R. Misra, S. Acharya, and S. K. Sahoo, Cancer nanotechnology: Application of nanotechnology in cancer therapy. *Drug Discov. Today* 15, 842 (2010).
 154. L. O. Cinteza, T. Y. Ohulchanskyy, Y. Sahoo, E. J. Bergey, R. K. Pandey, and P. N. Prasad, Diacyllipid micelle-based nanocarrier for magnetically guided delivery of drugs in photodynamic therapy. *Mol. Pharm.* 3, 415 (2006).
 155. Z.-L. Chen, Y. Sun, P. Huang, X.-X. Yang, and X.-P. Zhou, Studies on preparation of photosensitizer loaded magnetic silica nanoparticles and their anti-tumor effects for targeting photodynamic therapy. *Nanoscale Res. Lett.* 4, 400 (2009).
 156. R. Zhang, C. Wu, L. Tong, B. Tang, and Q.-H. Xu, Multifunctional core-shell nanoparticles as highly efficient imaging and photosensitizing agents. *Langmuir* 25, 10153 (2009).
 157. C.-T. Poon, P.-S. Chan, C. Man, F.-L. Jiang, R. N. S. Wong, N.-K. Mak, D. W. J. Kwong, S.-W. Tsao, and W.-K. Wong, An amphiphilic ruthenium(II)-polypyridyl appended porphyrin as potential bifunctional two-photon tumor-imaging and photodynamic therapeutic agent. *J. Inorg. Biochem.* 104, 62 (2010).
 158. T. Zhao, H. Wu, S. Q. Yao, Q.-H. Xu, and G. Q. Xu, Nanocomposites containing gold nanorods and porphyrin-doped mesoporous silica with dual capability of two-photon imaging and photosensitization. *Langmuir* 26, 14937 (2010).
 159. S.-H. Cheng, C.-H. Lee, C.-S. Yang, F.-G. Tseng, C.-Y. Mou, and L.-W. Lo, Mesoporous silica nanoparticles functionalized with an oxygen-sensing probe for cell photodynamic therapy: Potential cancer theranostics. *J. Mater. Chem.* 19, 1252 (2009).
 160. G. R. Reddy, M. S. Bhojani, P. McConville, J. Moody, B. A. Moffat, D. E. Hall, G. Kim, Y.-E. L. Koo, M. J. Woolliscroft, J. V. Sugai, T. D. Johnson, M. A. Philbert, R. Kopelman, A. Rehemtulla, and B. D. Ross, Vascular targeted nanoparticles for imaging and treatment of brain tumors. *Clin. Cancer Res.* 12, 6677 (2006).
 161. B. M. Barth, E. I. Altinoglu, S. S. Shanmugavelandy, J. M. Kaiser, D. Crespo-Gonzalez, N. A. Di Vittore, C. McGovern, T. M. Goff, N. R. Keasey, J. H. Adair, T. P. Loughran, D. F. Claxton, and M. Kester, Targeted indocyanine-green-loaded calcium phosphosilicate nanoparticles for *in vivo* photodynamic therapy of leukemia. *ACS Nano* 5, 5325 (2011).
 162. S.-H. Cheng, C.-H. Lee, M.-C. Chen, J. S. Souris, F.-G. Tseng, C.-S. Yang, C.-Y. Mou, C.-T. Chen, and L.-W. Lo, Trifunctionalization of mesoporous silica nanoparticles for comprehensive cancer theranostics—the trio of imaging, targeting and therapy. *J. Mater. Chem.* 20, 6149 (2010).
 163. H. Koo, H. Lee, S. Lee, K. H. Min, M. S. Kim, D. S. Lee, Y. Choi, I. C. Kwon, K. Kim, and S. Y. Jeong, *In vivo* tumor diagnosis and photodynamic therapy via tumoral pH-responsive polymeric micelles. *Chem. Commun.* 46, 5668 (2010).
 164. C.-W. Lai, Y.-H. Wang, C.-H. Lai, M.-J. Yang, C.-Y. Chen, P.-T. Chou, C.-S. Chan, Y. Chi, Y.-C. Chen, and J.-K. Hsiao, Iridium-complex-functionalized Fe₃O₄/SiO₂ core/shell nanoparticles: A facile three-in-one system in magnetic resonance imaging, luminescence imaging, and photodynamic therapy. *Small* 4, 218 (2008).
 165. P. Huang, Z. Li, J. Lin, D. Yang, G. Gao, C. Xu, L. Bao, C. Zhang, K. Wang, H. Song, H. Hu, and D. Cui, Photosensitizer-conjugated magnetic nanoparticles for *in vivo* simultaneous magnetofluorescent imaging and targeting therapy. *Biomaterials* 32, 3447 (2011).
 166. T. D. Schladt, K. Schneider, M. I. Shukoor, F. Natalio, H. Bauer, M. N. Tahir, S. Weber, L. M. Schreiber, H. C. Schroder, W. E. G. Muller, and W. Tremel, Highly soluble multifunctional MnO nanoparticles for simultaneous optical and MRI imaging and cancer treatment using photodynamic therapy. *J. Mater. Chem.* 20, 8297 (2010).
 167. J. R. McCarthy, Multifunctional agents for concurrent imaging and therapy in cardiovascular disease. *Adv. Drug Deliver. Rev.* 62, 1023 (2010).
 168. G. M. Lanza, X. Yu, P. M. Winter, D. R. Abendschein, K. K. Karukstis, M. J. Scott, L. K. Chinen, R. W. Fuhrhop, D. E. Scherrer, and S. A. Wickline, Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: Implications for rational therapy of restenosis. *Circulation* 106, 2842 (2002).
 169. J. Sun, X. Li, H. Feng, H. Gu, T. Blair, J. Li, S. Soriano, Y. Meng, F. Zhang, Q. Feng, and X. Yang, Magnetic resonance

imaging of bone marrow cell-mediated Interleukin-10 gene therapy of atherosclerosis. *PLoS One* 6, 1 (2011).

170. X. Hua, P. Liu, Y.-H. Gao, K.-B. Tan, L.-N. Zhou, Z. Liu, X. Li, S.-W. Zhou, and Y.-J. Gao, Construction of thrombus-targeted microbubbles carrying tissue plasminogen activator and their *in vitro* thrombolysis efficacy: A primary research. *J. Thromb. Thrombolys.* 30, 29 (2010).
171. J. N. Marsh, A. Senpan, G. Hu, M. J. Scott, P. J. Gaffney, S. A. Wickline, and G. M. Lanza, Fibrin-targeted perfluorocarbon nanoparticles for targeted thrombolysis. *Nanomedicine* 2, 533 (2007).
172. L. L. Ma, M. D. Feldman, J. M. Tam, A. S. Paranjape, K. K. Cheruku, T. A. Larson, J. O. Tam, D. R. Ingram, V. Paramita, J. W. Villard, J. T. Jenkins, T. Wang, G. D. Clarke, R. Asmis, K. Sokolov, B. Chandrasekar, T. E. Milner, and K. P. Johnston, Small multifunctional nanoclusters (nanoroses) for targeted cellular imaging and therapy. *ACS Nano* 3, 2686 (2009).
173. J. R. McCarthy, F. A. Jaffer, and R. Weissleder, A macrophage-targeted theranostic nanoparticle for biomedical applications. *Small* 2, 983 (2006).
174. Y. T. Lim, M. Y. Cho, B. S. Choi, Y.-W. Noh, and B. H. Chung, Diagnosis and therapy of macrophage cells using dextran-coated near-infrared responsive hollow-type gold nanoparticles. *Nanotechnology* 19, 1 (2008).
175. C. Wang and J. Irudayaraj, Multifunctional magnetic-optical nanoparticle probes for simultaneous detection, separation, and thermal ablation of multiple pathogens. *Small* 6, 283 (2010).
176. V. P. Zharov, K. E. Mercer, E. N. Galitovskaya, and M. S. Smeltzer, Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. *Biophys. J.* 90, 619 (2006).
177. W.-C. Huang, P.-J. Tsai, and Y.-C. Chen, Multifunctional Fe₃O₄@Au nanoeegs as photothermal agents for selective killing of nosocomial and antibiotic-resistant bacteria. *Small* 5, 51 (2009).

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