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EXPERT REVIEW

Polymeric Materials for Theranostic Applications

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ABSTRACT Nanotechnology has continuously contributed to the fast development of diagnostic and therapeutic agents. Theranostic nanomedicine has encompassed the ongoing efforts on concurrent molecular imaging of biomarkers, delivery of therapeutic agents, and monitoring of therapy response. Among these formulations, polymer-based theranostic agents hold great promise for the construction of multifunctional agents for translational medicine. In this article, we reviewed the state-of-the-art polymeric nanoparticles, from preparation to application, as potential theranostic agents for diagnosis and therapy. We summarized several major polymer formulas, including polymeric conjugate complexes, nanospheres, micelles, and dendrimers for integrated molecular imaging and therapeutic applications.

KEY WORDS drug delivery · imaging · nanoparticle · polymer · theranostics

INTRODUCTION

Nanotechnology in biomedical application has advanced rapidly over the past few decades, and it bears merits to revolutionize diagnosis and therapy in many diseases, such as cancer, arthritis, HIV, *etc.* (1). For therapeutic purpose, nanoscale formulations, such as Doxil® and Abraxane®, have been approved by the Food and Drug Administration (FDA) for clinical applications due to the increased drug efficacy and decreased systemic toxicity. Numerous promising nanoscaled drug formulations are also under active clinical evaluation for the treatment of various diseases (2,3)., Accompanying the development of nanomedicine, various molecular imaging devices have emerged as excellent tools for noninvasive, high sensitivity and high resolution detection at cellular and molecular levels. Each imaging modality has its unique advantages and disadvantages as summarized in Table I.

With the development of therapeutic and diagnostic techniques, there is an urgent need to combine the imaging function with therapeutic ability in one integrated platform so as to dynamically monitor the progress of diseases and therapeutic efficacy. In the conventional therapy, the progress of disease can hardly be monitored in real-time when medicine is being applied. The imaging section, if needed, has to be done separately. This divided therapy and imaging method is likely to compromise the optimal therapeutic time window to diseases, especially for malignant cancers, as well as result in higher cost and suffering to patients.

In line with this demand of multifunctional systems, a term 'theranostics' was coined to encompass the ongoing efforts to integrate molecular imaging and therapeutic agents into one system for clinical application (4,5). It is aimed to enable co-delivery of medicine and imaging agents in a single dose to bridges the gap between therapy and imaging to facilitate real-time monitoring the therapeutic efficacy of medicine. The nanoscale delivery system is a potential platform to realize the simultaneous molecular imaging and therapeutic purposes as required in the theranostic application. Traditionally, nanoparticles were investigated with single function, either for bioimaging or for therapy (Table II). Recently, nanoparticles have evolved to enable simultaneous imaging and therapy. Although it appears that the previous paradigms of single functional nanoparticles could be used directly to prepare theranostic platforms by including additional functionalities in the available delivery system (6), it should be noted that realization of coordinated diagnosis and therapy in the same regime of theranostics is not an easy task. Molecular imaging agents are expected to enhance signal to noise ratio at specific tissues. Such agents should present high tissue specificity, followed by relatively rapid clearance from

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Table I Summary of Commonly Used Molecular Imaging Modalities

Imaging modality	Imaging agent	Special resolution	Target	Advantages	Disadvantages
PET	^{18}F, ^{64}Cu, ^{11}C, ^{15}O labeled compounds	1–2 mm	Molecular, physiological	High sensitivity; imaging biochemical process; no depth limit; quantative results	High cost; Radiation; Low resolution
SPECT	^{99m}Tc, ^{111}In chelates	1–2 mm	Molecular, physiological	High sensitivity; no depth limit; relatively lower cost; quantative results	Radiation; Low resolution
MRI	Iron oxide; Gadolinium; Manganese oxide; ^{19}Fluorine labeled compounds	10–100 μm	Anatomical, physiological, molecular	High resolution; No radiation; no depth limit; quantative results	High cost; limited to patients with metallic implants
CT	Iodine	50 μm	Anatomical, physiological,	High resolution; relatively lower cost; no depth limit; low radiation exposure; quantative results	Need contrast agent for tissue differentiation at soft organ imaging; mainly used for lung and bone
Ultrasound	Microbubbles	50 μm	Anatomical, physiological,	High resolution; low cost, ease of operation; no radiation; quantative results	Limited imaging depth (cm)
Optical imaging	Fluorochrome; photoprotein	1–5 mm	physiological, molecular	High sensitivity; no radiation; multi-channel imaging	Not for clinical imaging, low resolution, limited imaging depth (<1 cm)

the animal/human body upon the completion of function. On the other hand, therapeutic medicine loaded nanoparticles are designed to have a relatively long circulation time in the body so that nanoparticles with drug could passively accumulate at the diseased site, such as tumor, and then the encapsulated drug can be released in a controlled manner. The discrepancy of bioavailability of nanoparticles for molecular imaging and for therapy necessitates the wise and careful design of theranostic nanomaterials.

There have been reports of a handful of successful theranostic nanoparticle agents in the literature. For example, chemotherapeutic agents have been conjugated onto gold nanoparticle surface for theranostic applications (7–10); iron oxide nanoparticles with appropriate surface modification have also demonstrated the potential for imaging guided therapy (11–13). Traditionally effective chemotherapeutic agents could be conjugated onto iron oxide nanoparticles for dual imaging and drug delivery purposes (13–15); quantum dots with their inherent fluorescent emission ability have been extensively investigated for bioimaging (16) and imaging guided therapies (17–19). Another widely investigated inorganic material, carbon nanotube, is also a good candidate for concurrent optical imaging and drug/gene delivery (20–25). All of the above mentioned nanoscale platforms in theranostics are inorganic/metallic nanoparticles. These nanoparticles, unfortunately, would be concerned by the inevitable toxicity, immunogenicity, and slow excretion kinetics from the body. Due to the excellent biocompatibility, biodegradability and structural versatility from natural to tailored synthetic sources, polymeric materials have played key roles in the development of drug formulations in the past, and more recently polymeric materials have become a hot pursuit for theranostic applications. Some representative polymeric materials as theranostic agents are shown in Fig. 1, and Table III summarizes the recent advances of polymeric materials in theranostic agent development.

POLYMER CONJUGATE COMPLEXES FOR THERANOSTIC APPLICATION

Differring from conventional polymeric matrix encapsulated drug delivery system, covalent conjugation of polymeric macromolecules with drugs or functional imaging agents is a new paradigm for drug and/or imaging agent delivery. The polymer conjugates cover a wide range from bioactive polymeric drugs (26,27), polymer-drug/imaging agent conjugates (28–32), to polymer-protein conjugates (33,34). Herein, we define the polymer conjugate as the one that the conjugated polymeric materials, either natural or synthetic, are employed to improve the solubility of poorly water soluble molecules, to enhance the bioavailability of drugs, or to orient agents to specific tissues.

For anticancer therapy, Ringsdorf's view of idealized polymer chemistry inspired the concept of targetable polymer-drug conjugates for cancer therapy (35). Small molecule drugs are likely to result in random distribution in the body, followed by notorious side-effects in cancer therapy,

Table II Examples of Polymeric Nanoparticles Used in Cancer Therapy or Imaging

Type of nanocarrier	Polymeric material	Drug	Current development status	Function	References
Polymer-drug conjugate	PGA	Paclitaxel	Phase III	Cancer therapy	(29,30)
	PGA	Camptothecin	Phase I	Cancer therapy	(33–35)
	PEG	Camptothecin	Phase II	Cancer therapy	(35)
	Hyaluronic acid	Paclitaxel	*In vivo*	Cancer therapy	(49)
	HPMA	Platinum	Phase I/II	Cancer therapy	(58)
	HPMA	Camptothecin	Phase I	Cancer therapy	(55)
	HPMA	TNP-470	Phase I	Cancer therapy	(68)
	Dextran	NIR dye/radio labeling	*In vivo*	Cancer therapy and imaging	(60)
Polymer nanosphere	PLGA	Docetaxel	*In vivo*	Cancer therapy	(87–90)
	PLGA	NIR dye and iron oxide	*In vivo*	Cancer imaging	(102)
Polymeric micelles	PLA-PEG	Paclitaxel	Phase I	Cancer therapy	(118)
	PCL-PEG	Docetaxel	*In vivo*	Cancer therapy	(119)
	PLA-PEG	TNP-470	*In vivo*	Cancer therapy	(121)
	PAA-PEG	Platinum	Phase I	Cancer therapy	(124)
Polymeric dendrimers	PAMAM	Paclitaxel/fluorophore	*In vivo*	Cancer therapy and imaging	(142)
	Polylysine	GO-DOTA	*In vivo*	Cancer imaging	(166)
	PAMAM	Gold nanoparticle	*In vivo*	Cancer imaging	(172)

HPMA N-(2-hydroxypropyl)methacrylamide; *PGA* polyglutamic acid; *PLA-PEG* poly(lactic acid)-poly(ethylene glycol); *PLGA-PEG* poly(lactic-co-glycolic acid)-poly(ethylene glycol); *PAA-PEG* poly(aspartic acid)-poly(ethylene glycol); *PAMAM* poly(amido amine)

while a suitable polymer-drug conjugate would prolong the circulation time of the drug, and facilitate passive tumor targeting through the leakiness of highly proliferating tumor vasculature by enhanced permeability and retention (EPR) effect (36). In addition, the polymer backbone of this conjugate can be tethered with targeting moieties, such as antibodies, peptides, or small molecules, to realize site specific delivery through receptor-mediated endocytosis pathways (37–41). Figure 2 represents typical examples of commonly used polymer-drug conjugates for therapeutic agent delivery.

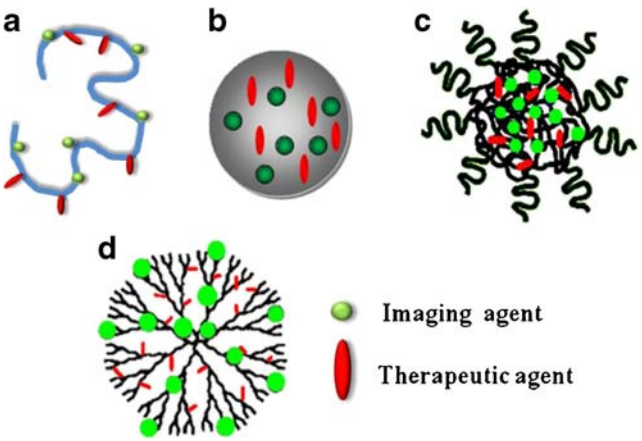

Fig. 1 Structural illustration of representative polymeric nanoparticles. (**a**) polymeric conjugate complex; (**b**) polymeric nanosphere; (**c**) polymeric micelle; (**d**) dendrimer.

Protein or Peptide Conjugate Complexes for Theranostic Application

Proteins or peptides as naturally occurring polymeric biomaterials are good candidates as delivery vehicles. Abraxane®, a paclitaxel-albumin conjugated nanoscale (130–150 nm) formulation, has been used in clinic (42). It excelled the conventional Cremophore EL/ethanol based formulation with reduced systemic toxicity and less drug administration frequency. Another widely used anticancer drug, doxorubicin, was chemically conjugated with elastin-like polypeptide self-assembly into nanoparticles in aqueous condition with enhanced plasma circulation time and efficient cellular uptake (43). Similarly, the conjugation of doxorubicin with a novel chimeric polypeptide could self-assemble into a nanoscale delivery vehicle. The resulting polypeptide-doxorubicin conjugate had a four-fold higher maximum tolerated dose (MTD) than the free doxirubicin, and induced nearly complete tumor regression after a single dose administration (44). In addition, the intrinsic fluorescence of doxorubicin makes this drug an appropriate candidate as a theranostic agent for monitoring the dynamic drug release progress *in vitro* and *in vivo* (45).

Polyglutamic acid (PGA) has also been applied for polymer-drug conjugation. One of such conjugates is PGA-paclitaxel (CT 2103, Xyotax) which is now under clinical evaluation. In this conjugate, the paclitaxel was covalently linked with PGA through the 2'-OH position to gain a very water-soluble complex with high drug loading content (37% w/w) (46,47).

Table III Representatives of Polymeric Theranostic Nanoparticles

Type	Polymeric material	Therapeutic agent	Imaging agent	Indication	Particle size scale
Conjugate complex	PGA	Mesochlorin e-6 (photosentisizer)	Gd-DOTA	MRI imaging and photodynamic therapy	~100 nm
	HPMA	Doxorubicin	Iodine-123	Gamma scintigraphy and chemotherapy	N.A.
	HPMA	Avastin®	Gadolinium	Biphasic administration therapeutic and imaging agents	N.A.
Nanosphere	PLGA	Doxorubicin	Iron oxide Nanoparticle	Concurrent drug delivery and MRI imaging	300–400 nm
	PGA	Doxorubicin	Polyfluorene containing oxadiazole (a fluorescent polymer)	Fluorescent imaging and drug delivery	>200 nm
Micelle	PGA-PEG	Cisplatin	Gd-DTPA	Drug delivery and MRI imaging	<100 nm
	PLA-PEG	Doxorubicin	Iron oxide	Drug delivery and MRI imaging	~150 nm
Dendrimer	PAMAM	Fluorescein isothiocyanate	Paclitaxel	Fluorescent imaging and Drug delivery	N.A.

Paclitaxel can be released from the conjugate upon hydrolysis of the ester bond, followed by further polymeric backbone degradation upon endocytosis by lysosomal cathepsin B catalysis. The increased water solubility and decreased normal tissue exposure led to improved safety profile of the conjugate over the free drug (47–49). Another widely used anticancer drug, camptothecin, was conjugated to PGA through a Gly linker for improved water solubility and higher efficacy (50,51). A recent review has systematically summarized the chemistry, physicochemical properties, and therapeutic applications of PGA-based drug conjugate complexes (52). For molecular imaging, the biodegradability of PGA makes it a good candidate for conjugation with gadolinium (Gd) chelate as a macromolecular MRI contrast agent with efficient clearance from the body (53,54). Upon modification with targeting ligand, cyclic Arg-Gly-Asp-D-Phe-Lys [c(RGDfK)] peptide, PGA-Gd conjugate could detect angiogenic biomarker integrin αvβ3 with T_1-weighted MRI (55). For theranostic application, Li and co-workers reported the MR imaging of therapy induced tumor necrosis by consecutive administration of PGA-paclitaxel and PGA-Gd conjugates (56). Lu's group reported the contrast enhanced-MR imaging the efficacy of photodynamic therapy (PDT) on xenograft tumors by bifunctional PGA-photosentisizer/Gd double conjugate (57). It was found that the PGA conjugate preferentially accumulated in the tumor region due to the hyperpermeability of the tumor vasculature, resulting in enhanced tumor contrast for accurate localization and imaging by contrast enhanced (CE)-MRI (58). Other commonly available peptides include polylysine, poly(aspartic acid) (59,60) (Fig. 3a) and various chimeric polypeptides (17,44,61–63) have also been attempted for conjugation with either drugs or imaging agents for theranostic applications.

Natural Polymeric Conjugate Complexes for Theranostic Applications

In addition to proteins and peptides, naturally occurring polymers, such as polysaccharides, have also been applied to develop drug or imaging agent delivery systems.

Hyaluronic acid or hyaluronate (HA) is a glycosaminoglycan (~10^6 Da) that exists in living system as a major component of the extracellular matrix (64). Its receptor, CD44, has been found to be overexpressed on many cancer cells (65). It is, therefore, a good polymeric material for disease targeted delivery of drugs or contrast agents (66). A novel HA solubilization method was used to conjugate paclitaxel to HA backbone with the addition of poly(ethylene glycol) (PEG). The as-prepared HA-paclitaxel conjugate complex self-assembled into micelles that readily released the entire paclitaxel under acidic condition. The HA-paclitaxel conjugate presented high cytotoxicity to CD44-overexpressing cancerous cells over CD44 deficient cells, suggesting that this HA-paclitaxel conjugate could be used as a tumor targeting macromolecular therapeutic agent (67). In another report, HA was covalently linked with methotrexate (MTX) by an enzyme cleavable spacer for the treatment of osteoarthritis with reduced risk of side-effects of MTX (68). HA was also conjugated with exendin-4 for type-2 diabetes treatment (69) or linked with photosentisizer for PDT of cancer (70). For HA based drug delivery, a recent review has summarized novel HA derivatives and the state-of-the-art of HA-based therapeutic delivery systems (71). As an effective molecular imaging agent, hydrophobically modified HA conjugated with near-infrared (NIR) dye has shown preferred tumor accumulation and enhanced imaging signal in animals (72).

Other natural polysaccharides, including chitosan, dextran, alginate, have also been well studied for conjugation

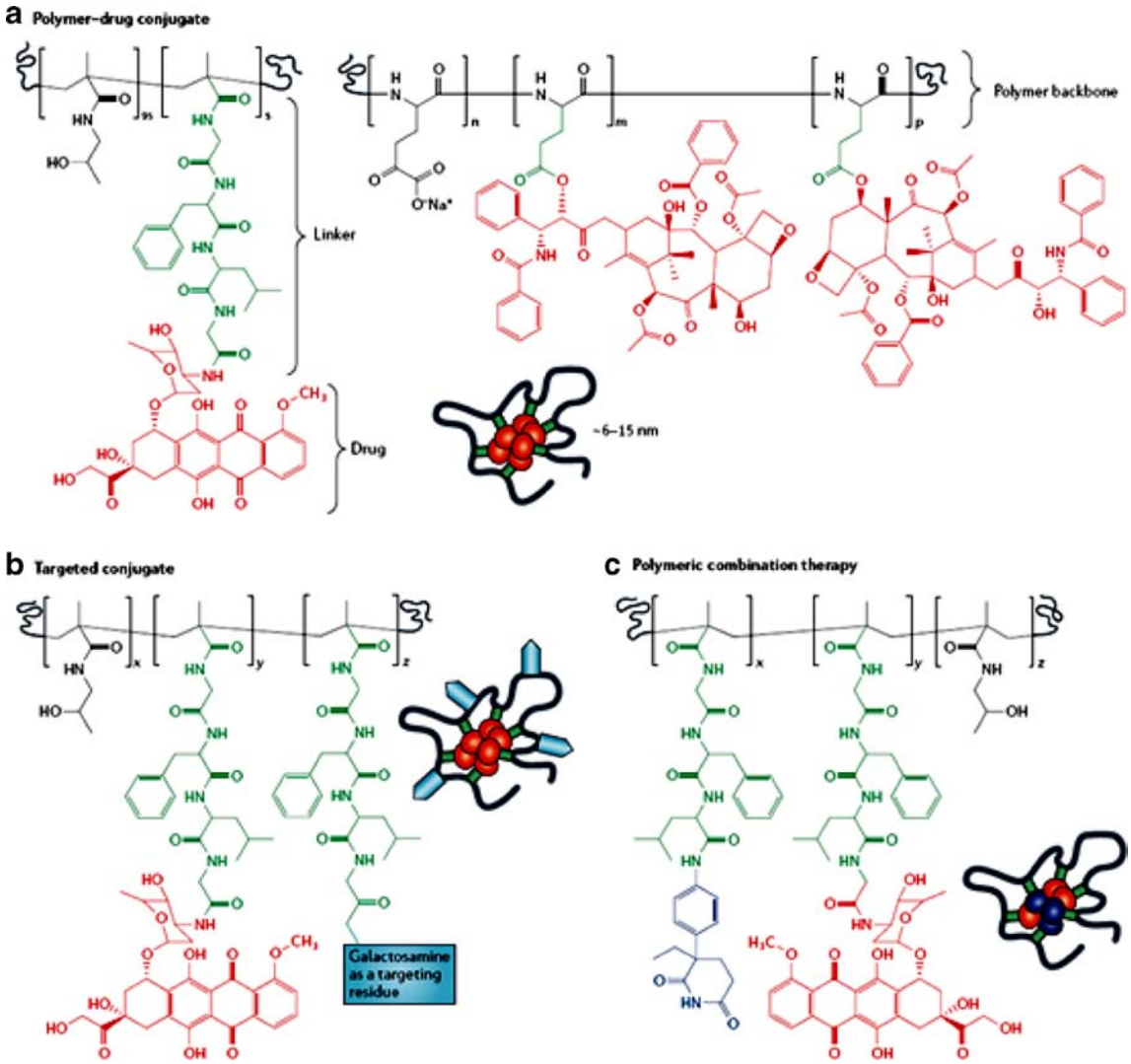

Fig. 2 Polymer–anticancer drug conjugates. Each panel shows both the detailed chemical structure and a cartoon of the general structure. The polymer backbone is shown in *black*, linker region in *green*, drug in *red* and additional components (for example, a targeting residue) in *blue*. (**a**) Two examples of more 'simple' polymer–drug conjugates containing doxorubicin (left) and paclitaxel (right) that have progressed to the clinic. (**b**) A multivalent receptor targeted conjugate containing galactosamine (*light blue*) to promote liver targeting. (**c**) Polymer combination therapy containing the aromatase inhibitor aminogluthethimide (*red*) and doxorubicin (*blue*) (adapted with permission from ref. (39)).

with drugs and/or imaging agents. It has been recently reported that galabiose-chitosan conjugate could be used for anti-*S. Suis* infection (73). In another research, photosensitive drugs and pH-responsive functional groups were added onto the backbone of glycol chitosan, followed by self-assembling in aqueous environment for anti-cancer therapy (74) (Fig. 3b). As an anti-inflammatory agent, sialyl Lewis X-chitosan conjugate was synthesized, which showed high binding affinity for E-selectin and potent inhibitory effect on the binding of E-selectin with SLe(x)-BSA (75). Low molecular weight hydroxyethyl chitosan (LMWHC) was conjugated to prednisolone (Pre) as effective potential drug candidate for the treatment of chronic renal disease (76). For the treatment of ulcerative colitis, an oral drug was fabricated based on the conjugation of budesonide with dextran as a polymeric prodrug. The *in vivo* result showed that conjugation of budesonide with 70 KDa dextran could decrease the macroscopic and microscopic scores of induced colitis compared with mesalasine and budesonide suspensions (77). As an imaging agent, the galactose modified dextran was coupled with Cy5.5 dye for NIR fluorescence imaging and radiolabeled with ^{99m}Tc for SPECT imaging (78). Dextran itself has also been labeled with ^{99m}Tc for angiocardiographical and/or lymphoscintigraphical imaging (79). Similarly, another polysaccharide, alginate, was tethered with drugs for anticancer therapy (80,81). However,

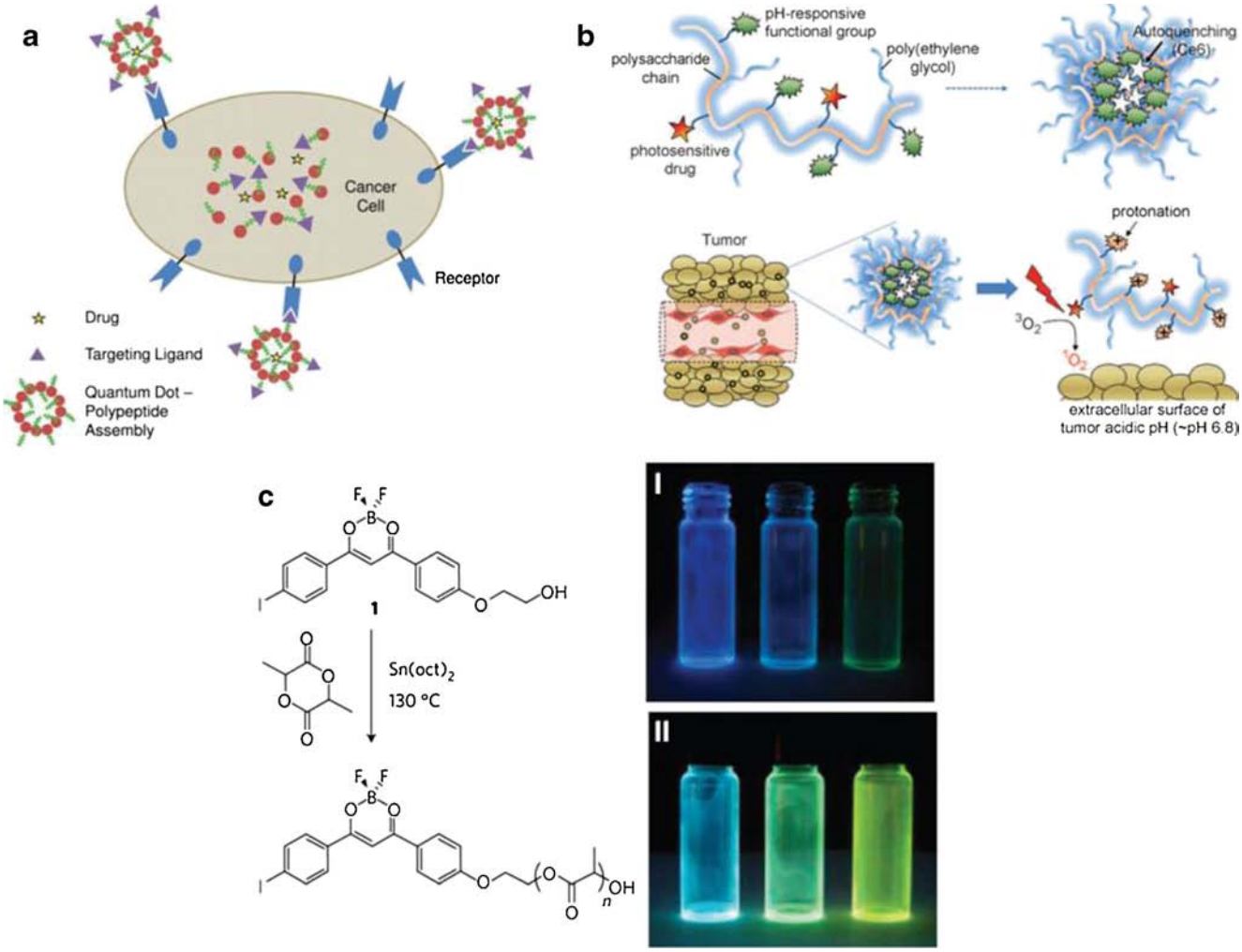

Fig. 3 Examples of polymeric conjugate complexes as theranostic agents. (**a**) Schematic of the application of quantum dot (QD)-polypeptide assemblies as dual imaging and targeted drug-delivery agents (adapted with permission from ref. (59)); (**b**) Schematic illustration of a proposed polysaccharide/drug conjugate. At high pH values, the conjugate complex undergoes autoquenching, and upon reaching the more acidic surface of the tumor cell protonation occurs and singlet oxygen is generated, thereby destroying the cell (adapted with permission from ref. (74)); (**c**) Synthesis and solid-state emission of BF2dbm(I)PLA under air (I) and N_2 (II) conditions (adapted with permission from ref. (99)).

due to the naturally high molecular weight of alginate, the application as a carrier for drugs or imaging agents is limited. In this regard, gamma irradiation has been used to reduce the molecular weight in addition to periodate oxidation treatment (82). A recent review has illustrated these polysaccharide-drug/imaging agent conjugate complexes for theranostic applications (83).

Synthetic Polymer Conjugate Complexes for Theranostic Applications

Despite the fact that naturally occurring versatile macromolecules can serve as delivery vehicles for theranostic applications, the inherent vulnerable characters, such as ease of hydrolysis or proteolysis, fast degradation of natural materials and their relatively simple structures necessitate the design of more sophisticated biocompatible materials for biomedical applications. Therefore, synthetic polymers play vital roles in the development of theranostic agents.

N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer has been extensively explored for numerous drug or molecular imaging agent delivery applications. HPMA is non-toxic, non-immunogenic, and stable in systemic circulation (71). HPMA drug/imaging agent conjugates have been studied for several decades. Some of the HPMA conjugates have been at different phases of clinical trials. Phase I evaluation of HPMA-Gly-Phe-Leu-Gly-doxorubicin (PK1) was initiated in 1994. The peptide linker is cleavable by lysosomal enzyme, cathepsin B, upon cellular uptake, while it is stable in the blood stream. It was reported to have four to five-fold higher maximum tolerated dose (MTD) than that of the free drug. By conjugating galactoamine to HPMA-peptide-doxorubicin conjugate

backbone, the conjugate complex could promote multivalent targeting of hepatocyte asialoglycoprotein receptor (ASGP-R) for the treatment of primary liver cancer (74). HPMA has also been conjugated with other conventional anticancer drugs, such as paclitaxel (PNU166945) (75) and camptothecin (MAG-CPT) (73) for cancer therapy. Unfortunately, clinical trials of these two types of HPMA drug conjugates showed negative results. However, HPMA-platinate conjugate (AP5280) demonstrated clinical success with reduced platinum related toxicity. An angiogenesis inhibitor TNP-470 was also conjugated with HPMA (Caplostatin) for anti-angiogenic treatment of cancer (84). It was found to selectively accumulate in the tumor vasculature, resulting in decreased tumor growth in two different cancer models. Interestingly, such HPMA-TNP-470 conjugate formulation does not cross the blood–brain barrier (BBB), thus overcomes the neurotoxicity of TNP-470 (84,85). As bone metastasis is highly associated with several types of solid cancers, such as breast cancer or prostate cancer, another novel bone-targeting HPMA chemotherapeutic drug conjugate complex has been constructed to prevent bone metastasis (86).

In molecular imaging, gadolinium-labeled HPMA conjugate has shown prolonged blood circulation time, hence, holds great potential for tumor diagnosis and monitoring (87). In addition, HPMA could be conjugated with gadolinium chelator, followed by further modification with c(RGDfK) peptide for integrin αvβ3 expressed on tumors or its microvasculature (88). Results demonstrated the potential of this conjugate as an effective targetable MRI contrast agent for tumor imaging and therapy monitoring. In another research, HPMA was conjugated with RGD4C, which was further radiolabeled with ^{99m}Tc for scintigraphic imaging. The HPMA-RGD4C conjugate showed prolonged tumor retention over 72 h and reasonably efficient clearance from normal organs and tissues (89). Being a theranostic agent, HPMA-doxorubicin conjugate was additionally introduced a small amount of methacryloyl tyrosinamide on the backbone for ^{123}I- or ^{125}I-labeling. This conjugate complex enabled tracing of time-dependent biodistribution of drug conjugate from two administration routes (intraperitoneal and intravenous) (90). To monitor the treatment efficacy of antiangiogenic drugs, a 40 k Da gadolinium-labeled HPMA copolymer (GDCC-40) was administered prior to the injection of VEGF-binding antibody Avastin®. This biphasic treatment was visualized by dynamic contrast enhanced (DCE)-MRI with the help of the macromolecular contrast agent HPMA-conjugate (91). A recent review has summarized the applications of HPMA in molecular imaging (31).

Both poly(lactic co-glycolic acid) (PLGA) and poly(lactic acid) (PLA) were traditionally used for drug delivery as the polyester matrix, where drugs were physically encapsulated. However, it was found that the burst release effect of encapsulated drug and low loading efficiency of hydrophilic drugs in the polyester matrices hindered the development of these materials as delivery vehicles for drugs and molecular imaging agents (92). To overcome such limitation, it has been proposed that direct conjugation of typical drug molecules or imaging agents onto PLGA or PLA polymers could reduce the burst release effect and enhance loading efficiency of hydrophilic drugs. In this regard, it was reported that the conjugation of doxorubicin to PLGA was achievable, and nanoparticle formulation resulted in sustained drug release with enhanced doxorubicin loading efficiency (93). It should be noted that the conjugated doxorubicin in the nanoparticle formulation showed less cytotoxicity than that of free doxorubicin to cancer cells. However, it is believed that long-term exposure of such doxorubicin-PLGA conjugate to cancer cells would present similar, if not better, therapeutic efficacy with reduced side effects. To improve the circulation time and active targeting ability of such doxorubicin-PLGA conjugate nanoparticles, the nanoparticle surface was further modified with poly(ethylene glycol) (PEG) and c(RGDfK) peptide to selectively target nanoparticles to integrin overexpressing cancer cells (94). Another research showed an acid-responsive drug delivery system by conjugating PLA-PEG with cisplatin derivatives through hydrazine bond, followed by nanoprecipitation method to form sub-100 nm nanoparticles. These nanoparticles could potentially minimize the drug loss during circulation in the blood, where the pH value is neutral, and trigger rapid intracellular drug release upon endocytosis by target cells (95). Alendronate (AE), a drug commonly used for the treatment of osteoporosis, was conjugated with PLGA *via* amide bond. This AE-PLGA conjugate complex demonstrated enhanced bone seeking ability than the naïve PLGA, had an acceptable degree of blood compatibility, and was not cytotoxic. Therefore, it is suitable for intravenous administration for osteoporosis treatment (96).

To deliver nanoparticles into the cytoplasm, PLGA was either conjugated with fluorescein or biotin molecule on the segment distal end, which could be further labeled for molecular imaging purpose (97). In one report, ^{99m}Tc-labeled PLGA nanoparticles enabled imaging of sentinel lymph nodes in Wistar rats. The as-developed ^{99m}Tc-PLGA nanoparticles provided a proof-of-concept for PLGA-based system as an advantageous alternative to currently used sentinel lymph node detection tools (78). Similarly, PLGA nanoparticles could be surface conjugated with chelating ligands, diethylenetriaminepentaacetic acid (DTPA) or 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA), for Gd^{3+} labeling and T_1-weighted MRI measurement (79). When a boron dye was conjugated with PLA *via* ring-opening polymerization method, the fluorescent emission from boron dye was adjustable through the molecular weight of the conjugated PLA segment (98). This dye conjugate complex nanoparticle was also a dual-emissive nanoparticle, emitting both

fluorescence and phosphorescence in a single system. In addition, the boron dye was found to be highly sensitive to oxygen by heavy-atom exchange. All the characters enabled the application of such nanoparticle formula for tumor hypoxia detection *via* fluorescence and phosphorescence imaging (99) (Fig. 3c).

Summary

The conjugation of polymeric materials with drugs or contrast agents has presented unprecedented advantages by altering drug delivery mechanism to diseased cells, prolonging the blood circulation time of the drug molecules, accelerating the clearance rate of imaging agents from body upon the completion of imaging process, *etc*. Many of these polymer conjugate complexes are currently being bested in human, highlighting the potential of polymer-drug or polymer-imaging agent conjugates for modern biomedical diagnosis and therapy. However, we should also bear in mind some issues related to the conjugation. For example, the coupling will change the chemical structure of drug molecules, often resulting in compromised therapeutic efficacy. In addition, the conjugation of imaging agents to polymers may prolong the circulation time, leading to relatively high background and suboptimal contrast. Therefore, further studies are needed to minimize the impact of chemical modification to the potency of drug molecules and strike the balance between the requirement of controlled release of therapeutics molecules and required rapid clearance of the contrast agents after the imaging studies are accomplished, in order to realize the full theranostic potential of polymer conjuagates.

POLYMERIC NANOSPHERES FOR THERANOSTIC APPLICATIONS

Nanospheres are defined as colloidal solid particles consisting of macromolecular substances with particle size ranging from 10 to 1,000 nm with drug of interest either adsorbed on the particle surface or sequestered in the particle matrix. Polymeric nanoparticles can be made from synthetic polymers, including PLA and PLGA, polycarbolactone (PCL), polycarbonate, or from natural polymers such as chitosan and collagen, and may be used to encapsulate drugs or imaging agents without additional chemical modification. A wide variety of polymeric nanoparticle-generating technologies exist, and these technologies have been comprehensively summarized in the literature (100,101). Upon encapsulation into nanoparticle formulations, drugs present improved treatment efficacy, more efficient cellular internalization, prolonged circulation time *in vivo*, reduced degradation before reaching the targeted cells and sustained drug release as compared to the free drugs. Alternatively, imaging agents encapsulated in polymeric nanospheres could favorably accumulate at the diseased site for enhanced contrast, and controllable clearance rate from the body.

Polymeric Nanospheres for Therapy

In 1980, Couvreur *et al*. reported one of the earliest examples of polymeric drug delivery system for cancer therapy, where anticancer drugs were adsorbed onto polyalkylcyanoacrylate nanosphere (102). They revealed the release mechanism of the drug from the polymer matrix, and studied tissue distribution and drug efficacy in a tumor xenograft model. A biodegradable polymeric nanosphere employed to encapsulate a photosensitizer for cancer therapy was recently reported by Weissleder and co-workers (103). They formulated the photosensitizer, meso-tetraphenylporpholactol, with PLGA into a nanosphere. They reported that the as-prepared nanospheres were stable and non-toxic upon systemic administration. After cellular uptake, the photosensitizer was readily released from the nanosphere matrix and became highly phototoxic. Irradiation with visible light led to cell-specific killing of several cancer cell lines. *In vivo* experiments showed complete eradication of tumor burden in animal models. They envisioned that this photosensitizer loaded polymeric nanospheres with selective phototoxicity may have widespread applications in cancer therapy (103).

Polymeric nanospheres are also widely used as alternative vehicles to deliver conventional anticancer drugs, such as paclitaxel, camptothecin, daunorubicin, doxorubicin, and methotrexate, *etc*. For example, it is well-known that the conventional paclitaxel formulation is based on Cremophor EL/ethanol formulation. The severe side effects of this formulation are of serious concern. To employ polymeric nanosphere for paclitaxel delivery, there have been a good number of reports on PLGA or other biodegradable biomaterial nanosphere-based formulations (104–107). Similarly, other hydrophobic anticancer drugs have also been encapsulated into polymeric nanospheres for cancer therapy. The as-prepared drug loaded nanospheres can be surface-modified with different ligands for active targeting purpose. It was reported that wheat germ agglutinin (WGA) modified nanosphere could recognize DU-145 prostate cancer cells, which overexpress sialic acid and N-acetylglucosamine on the cell surface (108). WGA was also used to decorate PLGA nanosphere for specific chemotherapeutic drug delivery to Caco-2 colon cancer cells (109). Various biodegradable polymeric materials have also been synthesized to meet the versatile functional requirement in the fast growing area of drug delivery. One such example is poly(β-amino ester) (PAE) which was used to formulate hydrophobic drugs into nanosphere with pH-responsive feature (110).

As polymeric nanospheres bear high drug loading capacity by the polymeric matrix, they also allow co-delivery of

drug cocktails. In cancer therapy, it has been discovered that multiple drug resistance (MDR) proteins in many types of cancers hinder the therapeutic efficacy of anticancer drugs. Thus, specific blockage of MDR function *via* either MDR gene silencing by siRNA or MDR inhibitors was believed to enhance the anticancer treatment effects. It was demonstrated that polymeric nanosphere co-loaded MDR-1 silencing siRNA and paclitaxel had superior therapeutic effects on refractory tumors than that of the individual agent (siRNA or paclitaxel) in the formulation (111). The combined delivery of apoptotic signaling molecule C-6 ceramide and paclitaxel in polymeric nanosphere could also reverse the drug efflux from MDR cancer cells for enhanced chemotherapy effect (112). For antiangiogenic therapy, it has been proposed that the combined delivery of drugs for antiangiogenesis and anti-cancer purposes could present significant therapeutic effects for malignant cancer types (113). In polymeric nanospheres, the antiangiogenic drug, combretastatin A4 and the anti-cancer agent, paclitaxel, were co-encapsulated with nanosphere surface modified with c(RGDfK) (37). The targeted dual drug-loaded nanosphere achieved significant tumor growth suppression *in vivo* compared to the control. Histological results revealed that the targeted dual drug nanospheres led to dramatic tumor vasculature disruption, significant cancer cell apoptosis and cell proliferation inhibition in a tumor xemograft model.

Polymeric Nanospheres for Molecular Imaging

In addition to drug loading and delivery, polymeric nanoparticles can also serve as reservoirs to load different types of molecular imaging agents, such as inorganic nanoparticles and fluorophores in the matrices.

For fluorescence-based molecular imaging, semiconductor quantum dots (QDs) (16,18,113–115) hold great promise as the new generation fluorescent probes to image biological processes. Nevertheless, the inefficient delivery of QDs into live cell cytoplasm is the major limitation that hinders the progress of live cell monitoring and tracking at the subcellular level. It was recently reported that the biodegradable polymeric nanoparticle encapsulated, antibody modified QDs could be efficiently internalized into live cell cytoplasm, followed by releasing QD conjugates into the cytosol (116). This approach facilitates multiplexed labeling of subcellular structures inside live cells without the requirement of cell fixation or membrane permeation. As compared to conventional intracellular delivery techniques, this approach allows highly efficient cytoplasmic delivery of QDs with minimal toxicity to the cell. In a similar manner, a cationic core-shell colloid was fabricated to sequester QDs in the matrix for efficient live cell cytosol delivery, followed by subcellular labeling (117). In another recent research, a mini-emulsion technique was applied to prepare sub-100 nm polymeric nanoparticles incorporating a fluorescent dye and a photochromic spiropyran derivative. The resulting nanoparticles showed the spectral properties of both the fluorescent dye and spiropyran, thus, UV and visible light can be applied to modulate the fluorescence emission of fluorescent dye in nanoparticles, resulting in photoreversible fluorescent emission from the polymeric nanoparticle (118).

Polymeric nanoparticles are also widely used for magnetic resonance imaging (MRI) (13,119). The MRI contrast agent such as iron oxide nanoparticles, could be entrapped in the solid polymeric matrix. Necessary nanoparticle modification may be needed for tissue specific delivery of such agents (13). It was reported that the core-shell structure of glycol chitosan surface modified polymeric nanoparticle in which iron oxide was loaded could be used for selective liver cancer imaging after intravenous administration (120). Magnetic iron-cobalt (FeCo) nanoparticles were also encapsulated into biodegradable PLGA nanoparticle for enhanced liver tumor bioimaging application (121). Since some types of polymeric materials are inherently semiconducting materials, the polymeric materials could be directly used as imaging agent upon formation of nanoparticles. In line with this concept, there is a recent report regarding the semiconducting polymer dots as ultrabright fluorescent probes for biological imaging (122). These polymeric dots exhibit several important characteristics for experimentally demanding *in vitro* and *in vivo* fluorescence studies, such as their high brightness, fast emission rate, excellent photostability, nonblinking, and nontoxic features. They can effectively and specifically label cellular targets, such as cell surface markers in human breast cancer cells, without much nonspecific binding. These ultrabright nanoparticles present a new opportunity to apply versatile semiconducting polymers to various fluorescence measurements in biology and biomedicine (122). Using free radical polymerization method, a series of multivalent, functional polymer nanoparticles with diagnostic/imaging units and targeting ligands for molecular targeting were synthesized (123).

For multi-modal molecular imaging, NIR dye and fatty acid surface coated iron oxide were co-encapsulated into PLGA nanoparticles. The imaging agent embedded nanoparticles showed high accumulation at the tumor site (124). Nanoemulsion technique was also employed to prepare iron oxide nanoparticles with surface modification of a NIR fluorophore allowing both optical and MR imaging (125). ^{99m}Tc-labeled iron oxide encapsulated in organic polymer was also reported for SPECT/MR imaging (126). Lee *et al.* developed an iron oxide nanoparticle MR contrast agent coated in poly(aspartic acid) with particle size of 45 nm coupled with RGD peptide for integrin $\alpha_v\beta_3$ targeting and a chelator for ^{64}Cu labeling and PET imaging (127). Xie *et al.* prepared a human serum albumin (HSA) coated iron oxide agent (HSA-IONP) with triple-modality imaging capacity

(128). In this nanosystem, fluorescent dye, Cy5.5, and positron emitting radionuclide, ^{64}Cu, were coupled onto the surface of iron oxide which was already coated with dopamine. *In vitro* and *in vivo* studies showed relatively long circulation half-life, massive tumor accumulation, efficient extravasation, low macrophage uptake of the HSA-IONP particles. Different types of polymeric nanoparticles have also been developed for other combination of multimodality imaging studies such as optical/MR (129–132), PET/CT (133), and MR/CT (134) (Fig. 4).

Polymeric Nanospheres for Imaging Guided Therapy

In addition to the applications for combined drug delivery and multi-modal molecular imaging, polymeric nanospheres could also be used as theranostic agents for simultaneous molecular imaging and therapeutic drug delivery, due to their high loading capacity and efficiency.

Gold nanoparticles with surface plasmon resonance (SPR) in the NIR region are of great interest for imaging and therapy. Unfortunately, gold nanoparticles with NIR absorbance are typically larger than 50 nm, above the threshold size of 5–6 nm required for efficient renal clearance. To solve the problem, researchers have prepared biodegradable polymer/gold nanosphere complexes, in which individual gold nanoparticle with 4 nm in size were linked by biodegradable polymers into nanoclusters with NIR absorption capacity. On the other hand, the nanosphere could also act as a NIR activatable nanosphere for photothermotherapy (135). Serving as a reservoir matrix, polymeric material was used to co-encapsulate iron oxide nanoparticles for MR imaging and anticancer drug, doxorubicin, for real-time tumor imaging and therapy as a theranostic agent. The surface of the resulting theranostic nanosphere was further conjugated with anti-HER2 antibody for active targeting to specific cancer cells (136). In addition to incorporating imaging agents and bioactive drugs in the polymeric nanosphere matrix, it has been revealed that imaging agents could be embedded in a polymer core followed by surface deposition of another biodegradable polymeric layer containing cytotoxic drugs. This unique composition structure allows dynamic monitoring of drug release and therapeutic response concurrently (137). In addition to the encapsulation method, a polymeric nanosphere conjugate was prepared by electrostatic assembly of cationic fluorophore conjugated polymer and anionic poly(glutamic acid) (PGA) coupled with doxorubicin. In this system, the cationic polymer fluorescence was highly quenched by doxorubicin through electron transfer mechanism. Upon cellular uptake of the complex nanosphere, the PGA is hydrolyzed to release the drug, inducing the activation of polymer fluorescence (138). This enables dynamic imaging of drug release process with fluorescence microscopy, and possible *in vivo* fluorescence molecular imaging at the diseased site.

Summary

Polymeric nanospheres were traditionally employed as delivery vehicles for hydrophobic drug delivery. Unfortunately, the therapeutic efficacy of these drug delivery systems could hardly be evaluated without imaging the delivery process. With the fast development of modern molecular imaging techniques, various polymer nanospheres have been engineered for molecular imaging purposes based on their

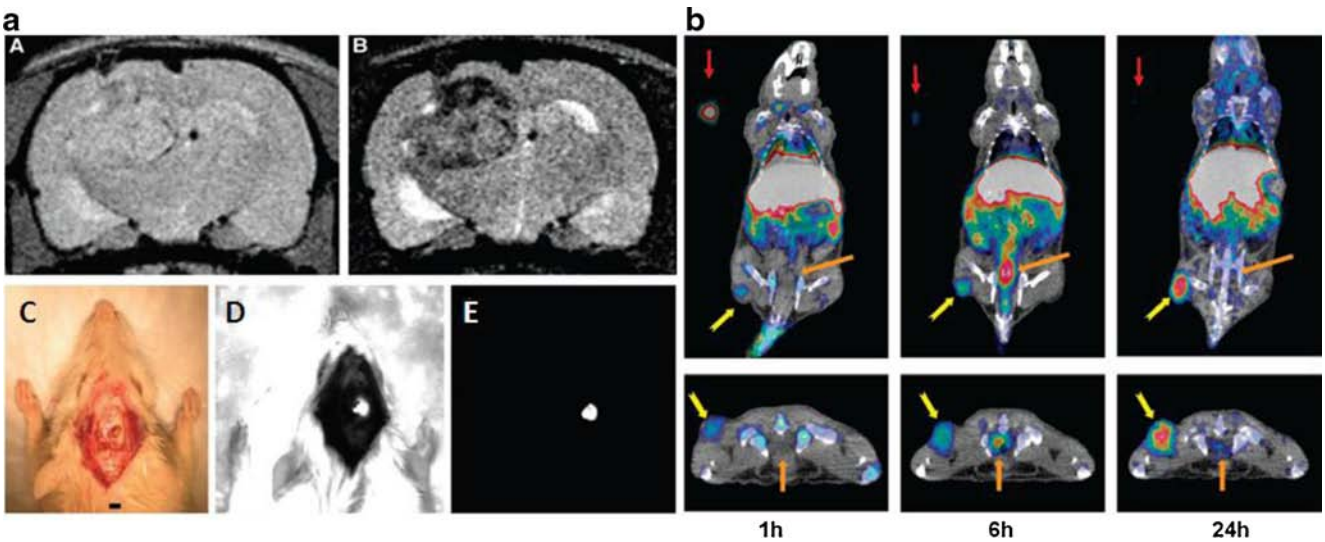

Fig. 4 (**a**) Cy5.5-CLIO nanoparticle as a preoperative MRI contrast agent (*A–B*) and NIR fluorescent imaging agent for tumor delineation (*C–E*) (adapted with permission from ref. (129)); (**b**) Micro-PET/CT images of nude mice bearing subcutaneous U87 glioma xenografts at different time points after i.v. injection of PEG-[^{64}Cu]CuS NPs (adapted with permission from ref. (133)).

advantageous biocompatibility, biodegradability, longer circulation time, fast and controllable clearance rate, *etc*. For theranostic applications, polymeric nanospheres also demonstrated their superiority of simultaneous loading of imaging agents and therapeutic drugs. Nevertheless, an ideal polymeric nanosphere-based theranostic agent requires efficient and sustained drug release at target site and rapid contrast enhancement followed by effective clearance after imaging. The widely used polymeric nanospheres, such as PLGA and PLA nanoparticles, may not meet these criteria. Thus, functionalization of traditional polymeric materials or synthesizing novel functional polymers specifically for theranostic agent delivery may point the future direction of polymeric nanosphere development in theranostic applications.

POLYMERIC MICELLES FOR THERANOSTIC APPLICATION

Polymeric micellar nanoparticles are composed of a variety of amphiphilic materials that can self-assemble into nanoscale particles upon interaction of hydrophobic segments between amphiphilic polymers and the hydrophilic portions to form a corona with or without functional groups at the distal end of polymeric chains. Thus, hydrophobic drugs or contrast agents can be entrapped into the micellar core through hydrophobic interaction or by covalent bonding with the polymer block comprising the hydrophobic domain. In contrast, charged hydrophilic macromolecules including peptides, proteins, and nucleic acids, can be loaded into the micellar core by using oppositely charged blocks to form polyion complexes through electrostatic interactions and charge neutralization. As corona-forming segments, several hydrophilic and non-ionic polymers, such as poly(ethylene glycol) (PEG), poly(N-vinyl pyrrolidone) (PVP), poly(N-isopropyl acrylamide) (PNIPAM), and poly(hydroxypropyl methacrylamide) (PHPMA), have been reported. Among them, PEG is the most commonly used hydrophilic block that confers micelles with biocompatibility, stealth-like properties, and site for functionalization (139).

Due to the ease of formulation, good stability, capacity of encapsulating hydrophobic molecules, polymeric micelles are widely investigated as viable drug and/or imaging agent delivery systems (140). For cancer therapy, Genexol-PM™, a formulation of paclitaxel encapsulated in a polymeric micelle, is currently under clinical evaluation. Several clinical trials have validated its safety and efficacy in metastatic breast cancer, non-small-cell lung cancer, and other solid tumor types (141). Many other biodegradable and biocompatible polymeric nanoparticles have also been synthesized to develop paclitaxel based micellar formulations. For instance, an amphiphilic poly(ethylene glycol)-co-poly(epsilon-caprolactone) (PEG-PCL) co-polymer was synthesized to load paclitaxel for controlled drug delivery (142). Another biodegradable poly(lactide-co-glycolide)-d-a-tocopheryl polyethylene glycol 1000 succinate (PLGA-TPGS) was also prepared by ring-opening polymerization method using TPGS as initiator. This co-polymer could load paclitaxel in high efficiency for long-term controlled anticancer drug delivery (143). For antiangiogenic and anticancer activities, a PLA-PEG based, TNP-470 loaded oral formulation (Lodamin) was developed. This oral drug formulation could be absorbed by the intestine and selectively accumulates in tumors. It was shown that Lodamin is a nontoxic antiangiogenic oral drug that can be chronically administered for primary tumor treatment or prevention of metastasis (144) (Fig. 5a). By using a chitosan derivative modified with long alkyl chains on the backbone, an amphiphilic N-octyl-O-glycol chitosan micelle loaded with paclitaxel was prepared as a promising drug carrier for injectable paclitaxel administration (145). In a similar manner, another natural macromolecule, hyaluronic acid (HA), was conjugated with hydrophobic oligomers on the backbone. This hydrophobic modification resulted an amphiphilic HA polymer that could load paclitaxel *via* polymer self-assembling progress in aqueous phase (146).

Metal-polymer chelating complexes have also been explored as micelle-based drug delivery platforms. The commonly used chemotherapeutic drugs, platinum complexes, such as cisplatin or carboplatin were incorporated into chelating polymers with amines as nitrogen donors and carboxylate/hydroxyl groups as oxygen donors through the ligand exchange of one or more ligand groups at the metal center. Polymeric micelles such as PEG–poly(aspartic acid) (PEG–PASP) and poly(glutamic acid) (PEG–PGA) block co-polymers have been extensively examined to incorporate platinum by complexation through ligand exchange between carboxyl groups in the poly(amino acid) block with chlorine or oxygen contained in the small molecule platinum complexes (147,148). The incorporated platinum complexes further enhanced hydrophobicity of the blocks and also cross-linked the blocks, leading to the formation of dense core within micelles.

To effectively deliver another wide-spectrum anticancer drug, doxorubicin, a charge-reversible micelle composed of poly(epsilon-carbolactone)-poly(ethyleneimine) (PCL-PEI) was prepared. The amine groups on PEI segment were converted into amides so that the entire drug loaded micelles were negatively charged at neutral pH but became positively charged at pH < 6 for efficient endosomal/lysosomal escape (149). As for pH responsive drug delivery, it was reported that a doxorubicin encapsulated nanogel consisting of a hydrophobic copolymer core and two layers of hydrophilic shells could infect tumor cells in a receptor-dependent manner, kill the cells, and migrate to neighboring cells like virus particles (150).

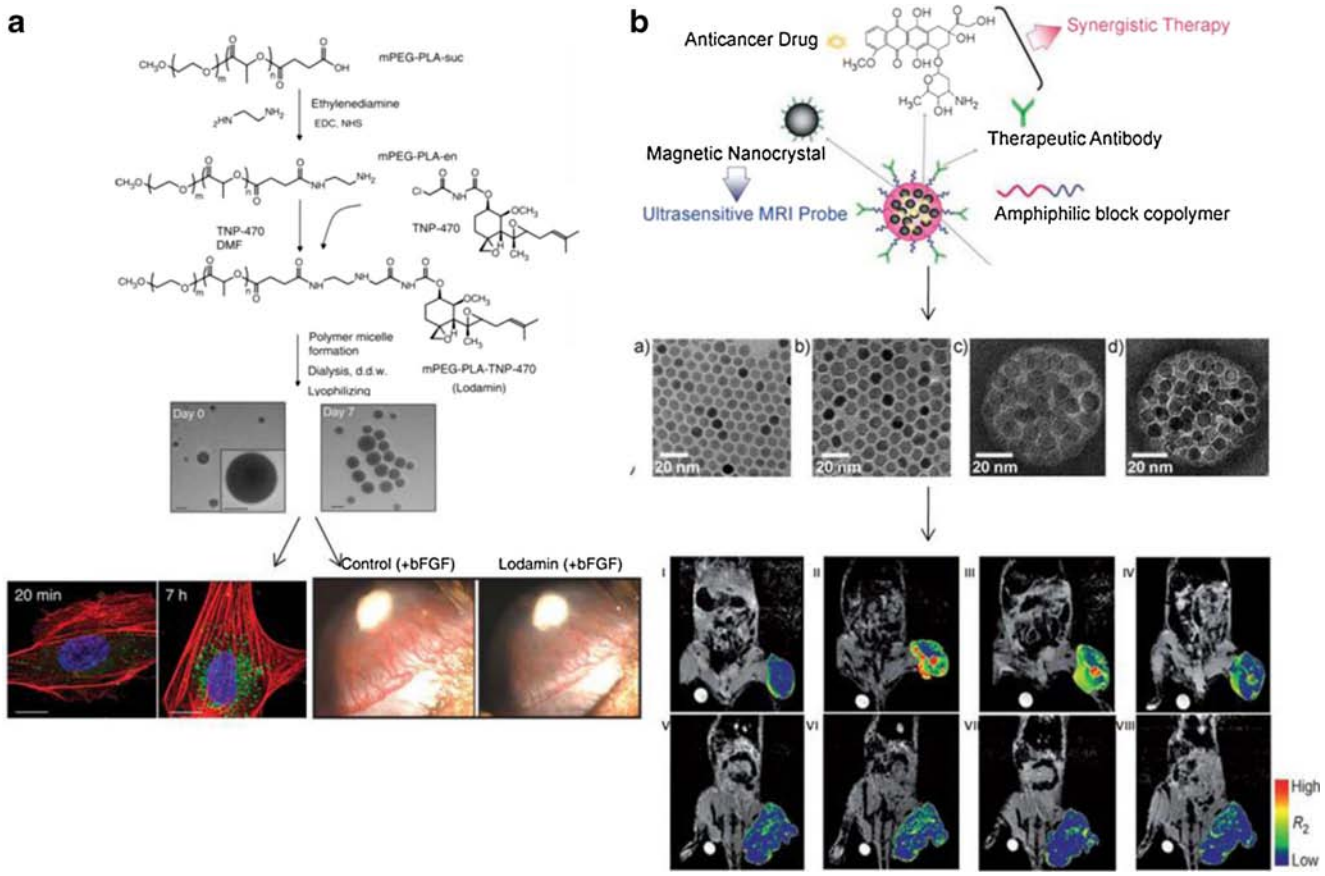

Fig. 5 Representative polymeric micelles as theranostic agents. (**a**) Upper channel: scheme of the conjugation reaction between TNP-470 and modified mPEG-PLA.; middle channel: TEM pictures of micelles at day 0 and day 7; bottom left: efficient HUVEC uptake of fluorophore labeled micelles; bottom right: corneal micropocket assay for the efficacy of that conjugate complex (Lodamin) to neovasculatures (adapted with permission from ref. (144)); (**b**) Upper channel: schematic illustration for the fabrication of magneto-polymeric nanohybrids (MMPNs); middle channel: TEM pictures of *a*) $MnFe_2O_4$ and *b*) Fe_3O_4 and MMPNs containing *c*) $MnFe_2O_4$ or *d*) Fe_3O_4 nanocrystals; bottom channel: MR images and their color maps (tumor region) of cancer-targeting events of HER-MMPNs (I–IV) and IRR-MMPNs (V–VIII) in NIH3T6 cells implanted in mice at various time intervals: (I, V) preinjection; (II, VI) immediately; (III, VII) 1 h; (IV,VIII) 12 h after injection of the MMPNs (adapted with permission from ref. (160)).

Micelles composed of poly(ethylene oxide) and poly(2-tetrahydropyranyl methacrylate) (PEO-b-PTHPMA) in aqueous solution could be disrupted by high-frequency ultrasound (1.1 MHz). This micelle could be utilized for environment triggered drug delivery (151). Thermoresponsive polymeric micelles provide an alternative for efficient anticancer drug delivery and alleviate possible toxicity to healthy cells (152). Other stimuli responsive micelles for drug delivery purposes have been well summarized in the literature (153–155), interested readers are recommended to refer to those reviews for more details.

In addition to therapeutic applications, contrast-loaded micelles can also be used for visualizing numerous organs, tissues and diseased sites. It was reported that a pH-responsive polymeric micelle encapsulating iron oxide nanoparticles was used as an acid-targeting MRI contrast agent for pathologic diagnosis. This MRI probe remained in a micellar state at neutral pH, while it could be disrupted in acidic pathological areas, followed by exposure of iron oxide contrast agent for MR imaging (156). In another research, MRI contrast agent, Gd-DOTA, was conjugated with the amine groups of poly(ethylene glycol)-polylysine (PEG-PLL) co-polymer, followed by addition of polyanion as counterion to prepare polyion complex micelles. In an animal study, the resulting micelles accumulated in tumor tissues, and MRI study showed that T_1 image of axial slice of tumor area was significantly enhanced at 24 h after the injection (157).

Importantly, polymeric micelles make it possible for theranostic application due to their high loading efficiency, structural flexibility, and ease of preparation. Kataoka and co-workers have reported the polymeric micelle vehicle incorporating gadolinium-based MRI contrast agents and platinum (Pt) anticancer drugs through reversible metal-chelation interaction (158). Similarly, Gao's group reported another theranostic micelle system in which MRI contrast agent, iron oxide, and anticancer drug, doxorubicin, were co-encapsulated into a biodegradable micelle composed of PEG-PLA co-polymer. To orient this theranostic micelle

specifically to cancerous cells, the surface of this micelle was decorated with RGD peptide. *In vitro* MRI and cytotoxicity studies demonstrated ultrasensitive MRI imaging and integrin $\alpha_v\beta_3$ specific cytotoxic response of this multifunctional polymeric micelle, which holds great promise for clinical theranostic applications (159). As a single theranostic platform, a magneto-polymeric multifunctional nanoparticle has been synthesized using ultrasensitive $MnFe_2O_4$ nanocrystals and chemotherapeutic agents by amphiphilic block copolymers for targeted detection by MRI and treatment of breast cancer (160) (Fig. 5b). The resulting theranostic nanoparticles demonstrated the ability of inhibiting tumor growth and ultrasensitive MR imaging. This theranostic model could also be extended to other cancer types or disease treatment with necessary technical adjustment. It was recently reported that a flexible hollow nanoparticle, self-assembled from poly(N-vinylimidazole-co-N-vinylpyrrolidone)-g-poly(D,L-lactide) graft copolymers and methoxyl/functionalized-PEG-PLA diblock copolymers, as an anticancer drug carrier for cancer targeting, imaging, and therapy (161). This multifunctional hollow nanoparticle exhibits a specific on-off switch drug release behavior, owing to the pH-sensitive structure of imidazole, to release drug in acidic surroundings (intracellular endosomes) and to capsulate drug in neutral surroundings (blood circulation or extracellular matrix). This unique feature made this multifunctional appropriate candidate for dynamic drug release monitoring application. For combined ultrasound tumor imaging and cancer chemotherapy, a biodegradable co-polymer was synthesized to stabilize perfluoropentane (PFP) nano/microbubbles, together with encapsulating anticancer drug, doxorubicin. The resulting multifunctional nanoparticles are tumor-targeted drug carriers, long-lasting ultrasound contrast agents, and enhancers of ultrasound mediated drug delivery (162).

In summary, the high loading capacity and structural flexibility of polymeric micelles have drawn much attention in the rapid development of nanotechnology. It is a challenge to maintain the structural stability of polymeric micelle by dramatic blood fluid dilution after intravenous administration. It is suggested that reversibly cross-linking the core of micelles could, to some extent, overcome this stability problem. More efforts are still needed to increase the micelle stability while maintaining high drug delivery efficiency in developing future polymeric micelle based theranostics.

DENDRIMERS FOR THERANOSTIC APPLICATION

Dendrimers are polymeric materials with hyperbranched nanostructures. The size of dendrimer can be tailored by controlling the number of polymerization generations. As polymerization progresses, a small, planar molecule initiator grows into a spherical nanostructure with cavities that therapeutics and contrast agents can be grafted. The final dendrimer molecular weight and chemical composition could be precisely controlled during the polymerization process. Those features facilitate individualized nanomedicine in theranostic agent development.

As a therapeutic agent itself, a specially designed dendrimer is under evaluation as a microbicide to prevent HIV and HSV infections (163). In anticancer therapy, it was reported that a generation 6 (G6) poly-L-lysine (PLL) dendrimer could act as an effective antiangiogenic therapeutic agent leading to solid tumor growth arrest (164). In that report, different animal models were used to evidence the antiangiogenic feature of the PLL dendrimer.

As multiple functional groups are available on a dendrimer backbone, many moieties could be conjugated or loaded onto the dendrimer for various purposes. It was reported that G5 poly(amido amine) (PAMAM) was conjugated with anticancer drug paclitaxel, fluorescent imaging agent fluorescein isothiocyanate (FITC), and a targeting ligand folic acid, as an integrated system for cancer cell targeted fluorescence imaging guided anticancer drug delivery (165). As in many cases, the potential toxicity of dendrimer is a serious concern for its clinical use. To overcome this, a biodegradable dendrimer was synthesized with Br-76 labeling for noninvasive angiogenesis imaging. A well-established angiogenesis marker targeting ligand, c(RGDfK) was also conjugated onto the dendrimer backbone for improved specificity (166). Another tumor targeting ligand, vascular endothelial growth factor (VEGF), has been conjugated with a boronated dendrimer for VEFG receptor targeting in neutron capture therapy of cancer (167).

In 1994, Wiener and coworkers were the first to validate the feasibility of using dendrimers chelated with metal ions as MRI contrast agents (168). Also, many conventional imaging agents, such as Gd-DOTA, could also be covalently grafted onto the dendrimer backbone for molecular imaging (169,170). In addition to the covalent conjugation and metal chelation, dendrimers are also widely used as coating layers to encapsulate inorganic nanoparticles for molecular imaging, due to the enhanced interaction of nanoparticles with dendrimer outer layers (171). It was demonstrated that acetylated dendrimer-entrapped gold nanoparticles were suitable for *in vitro* and *in vivo* computed tomography (CT) imaging of cancer cells (172). Fluorescent quantum dots could also be entrapped within folic acid modified dendrimer for imaging folate receptor overexpressing cancer cells (173). Other inorganic imaging nanoparticles, such as iron oxide (173,174), carbon nanotube (175) could interact with different molecular weight dendrimers for enhanced cellular uptake for cellular tracking or imaging. As a theranostic agent, a fluorinated dendrimer was used for concurrent drug delivery and molecular imaging with ^{19}F MRI (176) (Fig. 6). In another recent research, an apoptosis induction protein drug, cytochrome c, was co-encapsulated with NIR dye into a novel synthetic water-soluble hyperbranched polyhydroxyl dendrimer. This

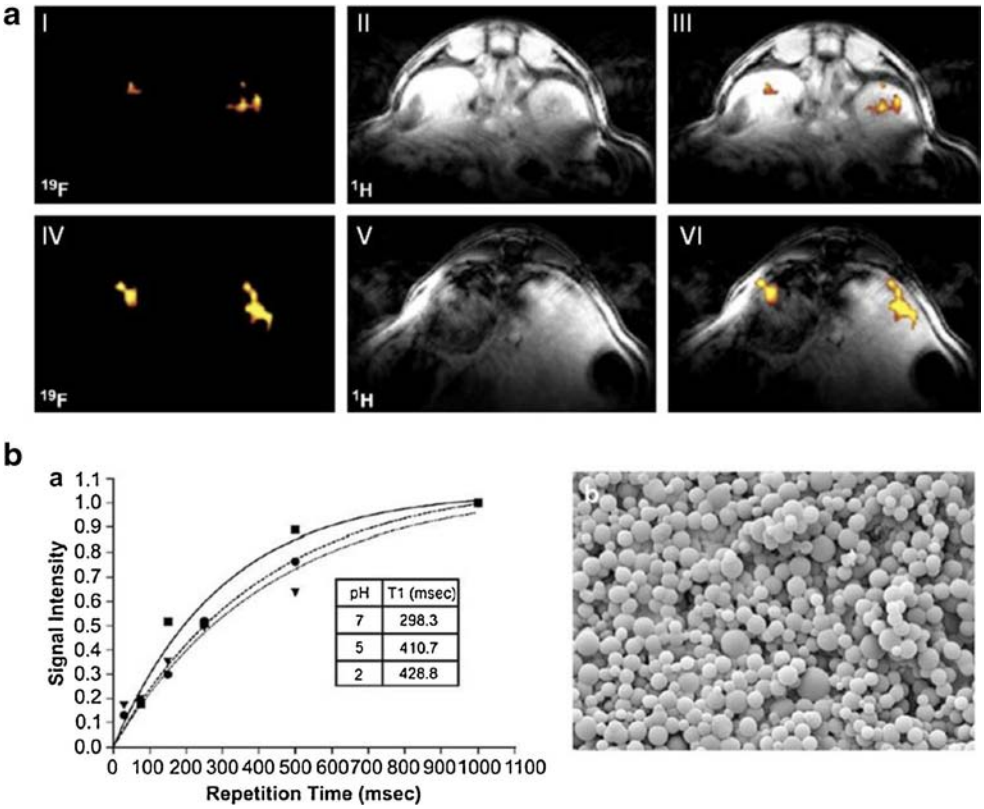

Fig. 6 (**a**) Noninvasive imaging with ^{19}F MRI. The inferior vena cava (IVC) of female B6 mouse was surgically catheterized just below the level of the liver to enable intravenous (i.v.) injection of 4 mg of PEGylated, fluorinated PAMAM (G3) particulates; (**b**) Fate of the fluorinated PAMAM(G3) particulates following exposure to low pH compartments: (*a*) pH-dependence of T1 relaxation times observed by ^{19}F magnetic resonance spectroscopy indicates a stable, rigid fluorine network within the particulate at physiological pH that is disrupted at low pH; (*b*) Scanning electron micrograph of fluorinated PAMAM (G3) particulates. (Adapted with permission from ref. (177)).

theranostic nanoparticle agent carrying an endogenous cellular apoptotic initiator (cytochrome *c*) and a fluorescent tag (ICG), was believed to hold promise for translation into the clinic (177).

Although dendrimers are widely used for the development of theranostic agents, their inherent toxicity arising from the multiple cationic ions can be a concern for clinical applications. It is suggested that the blockage of the unused cations by chemical modification may somehow lower the potential toxicity of dendrimers.

Another issue with dendrimer is the difficulty of purification. A dendrimer is assembled from a multifunctional core, which is extended outward by a series of reactions. Incompletion of the reaction will likely cause undesired trailing generations. Such impurities can impact the functionality and symmetry of the dendrimer, but are extremely difficult to purify out because the relative size difference between perfect and imperfect dendrimers is very small.

CONCLUSIONS

In this review article, we summarized the recent advances of polymeric materials aimed for sophisticated multifunctional theranostic agent development for biomedical application. The advantages of employing polymers as vehicles for theranostic agents are obvious. First of all, it was evidenced by numerous results that polymeric nanoparticles hold high capacity to sequester sufficient therapeutic compounds for disease treatment or contrast agents for improved imaging quality. Secondly, the structural versatility of widely available polymeric materials, from both natural and synthetic sources, facilitates nanoparticle functionalization for ligand directed active targeting or nanoparticle surface manipulation to reduce unfavorable protein-nanoparticle interaction for *in vivo* theranostic applications. Thirdly, many polymeric materials are biodegradable, biocompatible and of low toxicity, if any, to mammalian cells. These biologically friendly features also make polymeric nanoparticles attractive for translational theranostic agent development. Last but not least, polymeric nanoparticles could be combined with other materials, such as metallic nanoparticles or silica nanoparticles to form nanoscale complexes with diverse functions.

Up to now, polymeric nanoparticles have achieved a number of encouraging successes, and some of the polymeric materials based drug or imaging agent formulations are currently under clinical trials. However, the inherent disadvantages of polymeric nanoparticles, such as the vulnerability with hydrolysis or proteolysis, stability issues, should be paid much attention in our future design of polymeric material based theranostic agents.

Additionally, the design of theranostic agents with both therapy and imaging functions also suffer from numerous limitations. For example, the loading capacity of nanoparticles

may be challenged when therapeutic medicine and imaging agent are simultaneously loaded into the nanoformulations. Hence, the choice of optimal nanoformulation method is very critical in fabricating effective theranostic agents. For imaging purpose, many imaging agents, such as radioisotopes or fluorophores, are simply labeled or conjugated onto the polymer backbone. The pharmacokinetics (PK) and toxicity of these imaging agents labeled polymers have not been fully investigated. Furthermore, most imaging modalities in theranostic agents are not fully utilized, but just employed as simple evaluation tools for either diagnostic or prognostic purposes before or after therapeutics are administered. However, there is no guarantee that the imaging results could directly reflect the therapeutic outcomes. The correlation between imaging results and therapeutic efficacy needs to be investigated for reliable theranostic agent preparation. Also, the optimal time interval between molecular imaging and therapeutic intervention of diseases requires further understanding of the functions and characteristics of the theranostic agents.

As an integrated all-in-one platform, the development of theranostic agents points to the goal of individualized medicine, where drug and/or imaging doses could be designed according to individual requirement. Although only very few theranostic agents are currently in clinical trials, the cooperative combination of therapeutic and diagnostic agents in polymeric nanoparticles would significantly contribute to the clinical translation of theranostic agents for improved diagnosis and therapy to improve the health of human beings.

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