Review

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Dendrimers for theranostic applications

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Abstract: Recently, there have been tremendous advances in the development of various nanotechnology-based platforms for diagnosis and therapy. These nanoplatforms, which include liposomes, micelles, polymers, and dendrimers, comprise highly integrated nanoparticles that provide multiple functions, such as targeting, imaging, and therapy. This review focuses on dendrimer-based nanocarriers that have recently been developed for 'theranostics (or theragnosis)', a combination of therapy and diagnostics. We discuss the *in vitro* and *in vivo* applications of these nanocarriers in strategies against diseases including cancer. We also explore the use of dendrimers as imaging agents for fluorescence imaging, magnetic resonance imaging, X-ray computed tomography, and nuclear medical imaging.

Keywords: dendrimer; drug delivery; gene delivery; imaging; theranostic.

Introduction

The term 'theranostics' (or 'theragnosis'), which has recently been coined by combining 'therapy' and 'diagnostics', has attracted great attention. A single platform that integrates a therapeutic drug and an imaging agent is called a theranostic device. Theranostic devices offer the advantages of concurrent diagnosis and treatment for certain diseases. Because monitoring of the biodistribution and functions of the administered therapeutic agent, and detection of the target tissue are also important for therapy, theranostic nanomaterials that can simultaneously track and cure diseases have been intensively studied in the past few years (1). Historically, nanomaterials for therapy and imaging were developed independently. There are some nanomaterials based on polymers, micelles, and liposomes. Numerous polymeric nanodevices can be applied to drug and gene delivery against cancer, as well as to imaging and cell tracking through specific biomarkers and biosensors (2). Some linear polymers, for example, polyethylene glycol (PEG) and poly(lactic-co-glucolic acid) (PLGA), have been accepted in various clinical applications. However, there are some problems such as polydispersity, nonspecific biodistribution, and low loading capacity. Some drug-encapsulated liposomes and micelles are in clinical trials. However, liposomes and micelles are generally recognized and engulfed by phagocytic cells in the reticuloendothelial systems, resulting in low therapeutic index (3).

Dendrimers are synthetic macromolecules that have a highly branched spherical structure. In general, dendrimers have repeated branches of a dendron around a central core, resulting in a near-perfect three-dimensional geometrical shape (4). The size and molecular weight can be controlled by the generation (G). The dendrimer interior is available for the encapsulation of drug molecules; the peripheral functional groups are available for the modification of drug molecules through covalent bonds, and for complex formation. A great deal of attention has been paid to the development of dendrimers for various imaging platforms and drug delivery systems (5–18). Various dendrimers have been used to carry a variety of therapeutic drugs with the aim of maximum therapeutic efficacy in cancer treatment. The most studied anticancer chemotherapeutic drugs, including cisplatin, paclitaxel (TAX), doxorubicin (DOX), camptothecin, and methotrexate (MTX), have been formulated in dendrimer-based drug delivery vehicles. The dendrimers have also been used in gene delivery, vaccines, antiviral treatments, and diagnostic tools (19). An antiviral dendrimer, VivaGel, is currently in its phase I clinical trial for women to protect from genital herpes and HIV infection (20). Docetaxel-conjugated dendrimer formulation is also

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in clinical trial for advanced or metastatic cancer therapy (21). Similar to other nanomaterials, biocompatibility and tumor specificity can be added to dendrimer-based nanodevices by choosing proper chemical structures and proper modification at the periphery (21, 22). In addition, dendrimers possess the ability to facilitate the transport of therapeutics across various cell membranes or biological barriers via an endocytosis-mediated cellular internalization (22). Besides, controlled drug release from dendrimers can also be achieved by adding stimuli-sensitive moieties to the dendrimer, which allows administration of lower doses to lead to a decrease in toxicity against healthy cells. The advantages of dendrimers are its well-defined structures and multifunctionality. Dendrimers can work as a unimolecular nanoparticle (NP) with controllable size and chemical structure. This property is of benefit for chemical and biological reproducibility. Dendrimers have been recognized as the most versatile compositionally and structurally controlled nanoscale devices for nanomedicine. In general, 10- to 100-nm dendrimer nanodevices have been used as theranostic platforms (23). The multifunctionality of dendrimers allows a high payload of drugs and other bioactive molecules such as targeting ligands and imaging agents to the single dendrimer molecule through chemical modifications or in combination of conjugation, encapsulation, and complexation. Owing to these properties, dendrimers are suitable as nanoplatforms for theranostic devices.

Polyamidoamine (PAMAM) dendrimers, which are commercially available, were first reported by Tomalia et al. (1990). Several other dendrimers, such as poly(propyleneimine) (PPI) and poly(l-glutamic acid), have also been studied for their role in drug delivery (24). The glycopeptide dendrimers have been used in antitumor and antiviral prophylactic or therapeutic vaccines (25). Dendrimers with a polyhedral oligomeric silsesquioxane (POSS) core have been used for biomedical applications. The POSS core has certain structural advantages over traditional dendrimers. These POSS core dendrimers possess high surface functional group content, well-defined nanosizes, and compact globular macromolecular architectures. They have more peripheral functional groups because of the eight reactive termini of the POSS core. The high peripheral functionality helps binding of lipophilic anticancer drugs and imaging agents, as well as targeting agents (26).

In this review, dendrimer imaging agents for fluorescence imaging, magnetic resonance imaging (MRI), X-ray computed tomography (CT), and nuclear medicine imaging, such as positron emission tomography (PET), and single photon emission computed tomography (SPECT), are briefly discussed. The theranostic applications combining these imaging technologies with therapeutic treatments by using dendrimers are also indicated.

Theranostic dendrimers for fluorescence imaging

Fluorescence sensing technology has broad applications in diagnostics and the life sciences (27, 28). Although numerous synthetic organic fluorophores with sensing capabilities have been developed, an inability to target the desired cells or tissues, poor solubility, and uncontrollable intracellular localization are serious barriers to *in vivo* biomedical applications. The development of a dendrimer-based imaging agent is a possible solution to these drawbacks.

Majoros and coworkers (29) developed various kinds of multifunctional PAMAM dendrimers as theranostic anticancer devices. The PAMAM dendrimers contained the anticancer drug MTX, fluorescent dye fluorescein isothiocyanate (FI), and targeting ligand folic acid (FA) (29). The group also studied a saccharide-terminated G3 PAMAM dendrimer as a carrier for theranostic applications. The dendrimer conjugates showed efficient cellular internalization and cytotoxicity against the folic acid receptor (FR)-expressing KB cell line (30). They also reported that the multifunctional PAMAM dendrimer conjugate was prepared using click chemistry to obtain a target-specific theranostic anticancer device (31). The same group has also developed a dendrimer-based conjugate with the anticancer drug TAX and a fluorescent dye (Cy5), and has determined the mechanism of TAX action on microtubules. Using the single microtubule imaging technique, they demonstrated that the TAX-dendrimer conjugate binds to microtubules (32).

He et al. prepared a G5 PAMAM dendrimer conjugating PEG, an arginyl-glycyl-aspartic acid (RGD) peptide and FI (G5-FI-PEG-RGD). G5-FI-PEG-RGD was used as a multifunctional dendrimer for the delivery of DOX to integrin $\alpha_v\beta_3$-overexpressing glioblastoma U87MG cancer cells. Stable encapsulation was observed between the interior hydrophobic spaces of the dendrimers and DOX. The complex exhibited sustained release and specific internalization into cells via a receptor-mediated endocytosis pathway (33).

Dendrimer-based nanogels were developed by incorporating G5 amine-terminated PAMAM dendrimers and DOX into alginate nanogels (AG) using an emulsion method. The dendrimer was modified with FI and incorporated into the nanogels to prepare a macromolecular

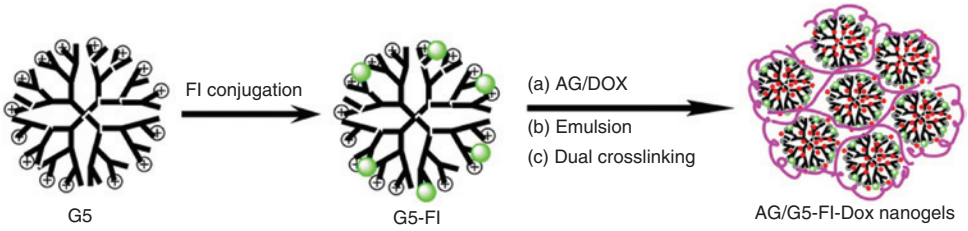

Figure 1: Preparation procedure of nanogels made from DOX-loaded fluorescent dendrimers and AG. Reproduced from Ref. (34). Copyright ©2014, American Chemical Society.

imaging agent (Figure 1). Because of the florescent nature of the DOX-loaded AG, the internalization into CAL-72 cells was evaluated through fluorescence microscopy. The fluorescent signal was observed in the cytoplasm treated with DOX-loaded nanogel, indicating that the nanogels accelerate the cellular uptake process. The authors showed that the sustained drug release ability of AG/G5-Dox resulted in the prolonged anticancer activity. The results demonstrated that AG/G5 nanogels are a promising platform for therapeutic delivery and/or bioimaging applications (34).

Minko and colleagues designed PAMAM dendrimer-based theranostic devices and evaluated their activity *in vitro* and *in vivo*. TAX, near-infrared (NIR) fluorescent dye (Cy5.5), and the targeting ligand (luteinizing hormone-releasing hormone [LHRH] peptide) were conjugated to the dendrimer. Because LHRH is overexpressed in the plasma membrane of breast, ovarian, and prostate cancer cells (35–37), the LHRH peptide can work as a ligand for tumor targeting. The dendrimer nanodevice showed high antitumor therapeutic activity with low adverse side effects on healthy organs, and high accumulation at the tumor. Because free Cy5.5 and dendrimers without ligands do not accumulate at the tumor, the design of the multifunctional dendrimer nanodevice was important for efficient theranostic activity (38).

FI-conjugated dendrimers have also been applied to small interfering RNA (siRNA) delivery. A cationic carbosilane dendrimer (G2) was modified with FI and then complexed with siRNA against the Nef protein to form a dendriplex. Because Nef silencing is known to interfere with HIV-1 infection, the dendriplex is applicable as an anti-HIV agent. By using FI, the uptake of dendriplexes by human primary astrocytes can be observed. The dendriplexes were inoculated into BALB/c mice by the retro-orbital venous plexus, which were detected in the brain from the *ex vivo* imaging. The presented results showed that delivery and transfection of siRNA to HIV-infected human primary astrocytes induced gene silencing without causing cellular toxicity. These results highlight the potential of the dendrimer formulation for the treatment of neurological disorders and the delivery of siRNA to the brain (39). Pan et al. reported that magnetic NPs (MNPs) covered with different generations of PAMAM dendrimers were complexed with antisense survivin oligoDNA (40). The gene transfection efficacy, the uptake mechanism, and the biological effects of the nanodevice against breast cancer cells (MCF-7 and MDA-MB-435) and liver cancer cells (HepG2) were evaluated. Furthermore, FI was attached to the dendriplex for the investigation of cellular entry of this device into various cell lines in a confocal microscopic imagining experiment. The results showed that PAMAM dendrimer-modified MNPs are a good gene delivery system and have potential applications in cancer therapy and molecular imaging diagnosis (40).

Photodynamic therapy (PDT) has become an efficient approach in clinical therapy for the noninvasive treatment of some affected cells and their visualization by fluorescence imaging (41). PDT involves administration of nontoxic photoactive drugs (photosensitizers). Upon exposure to light of a specific wavelength, the photoactive drug produces reactive oxygen species (ROS) that damage cancer cells. The dendrimer porphyrin (DP) has a porphyrin chromophore at the center and is surrounded by a framework of several aryl ether dendrites (42). DPs that can transport absorbed energy to the porphyrin center over relatively large distance via the dendritic architecture, and can effectively produce highly toxic singlet oxygen ($^1O_2$), are being used as photosensitizers for PDT (43). In such cases, the dendrimer is being used as the therapeutic agent only. Phthalocyanine (Pc) is a PDT photosensitizer with NIR optical properties, and also has potential in fluorescence imaging. However, its clinical applicability is limited because of its poor water solubility and insufficient selectivity to cancer cells. A Pc-based theranostic agent was prepared by the encapsulation of mono-substituted Pc into a G4 PPI dendrimer, followed by modification with PEG and synthetic LHRH peptide to improve its biocompatibility and tumor cell selectivity. The synthesized Pc-dendrimer

nanodevice exhibited a distinct NIR absorption (700 nm) and fluorescence emission (710 and 815 nm). The theranostic agent generated effective fluorescence emission and ROS upon laser activation to detect and damage tumor cells. The addition of PEG and LHRH peptide significantly diminished cytotoxicity and substantially enhanced cancer cell entry. Imaging experiments showed that the LHRH-targeted NIR theranostic dendrimer is capable of efficient *in vitro* and *in vivo* internalization into cancer cells, as well as tumor accumulation (44). Taratula et al. developed an effective naphthalocyanine agent-based theranostic nanoplatform, capable of concurrent NIR fluorescence imaging and combined phototherapy (45). Silicon 2,3-naphthalocyanine bis(trihexylsilyloxide) (SiNc) exhibited dual PDT and photothermal therapy (PTT) capabilities, but is water insoluble. In the study, a SiNc-loaded nanoplatform was prepared by encapsulation of SiNc into the interior of a G5 PPI dendrimer, which was further modified with PEG. Because the PPI dendrimer has hydrophobic pockets (46), the SiNc molecules were stably encapsulated and isolated. The encapsulation consequently decreased aggregation, enhanced water solubility, and preserved fluorescence intensity for imaging, PDT, and PTT. The developed dendrimer nanoplatform had good photo and thermal stabilities, which are essential for clinical applications. The authors demonstrated the remarkable combinatorial phototherapeutic effects of the platform in chemotherapy-resistant ovarian cancer *in vitro* and *in vivo*. The *in vivo* studies involved injecting the dendrimer nanoplatform intratumorally at the left tumor region. The obtained results demonstrated both the NIR imaging capability and the phototherapeutic efficacy of the dendrimer nanoplatform (Figure 2). The efficiency of the dendrimer nanodevice as an NIR imaging agent was confirmed by recording the strong fluorescence signal in the tumor area without photobleaching. No significant intensities were observed in the untreated control tumor. The antitumor activity was observed in the dendrimer nanoplatform-injected mice after laser irradiation (45).

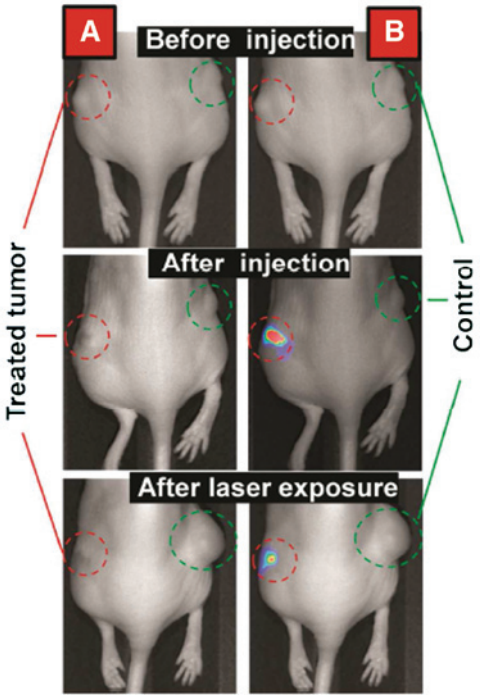

Figure 2: *In vivo* imaging and combinatorial phototherapy studies of SiNc-NP. Representative light (A) and fluorescence (B) images of tumor-bearing mouse before injection with SiNc-NP (top images), after injection (middle), and 25 days after treatment (bottom) with the laser diode (785 nm, 1.3 W/cm^2, 10 min). The left tumor was injected with SiNc-NP and exposed to the laser diode. The untreated right tumor was used a control. Reproduced from Ref. (45) with permission from the Royal Society of Chemistry.

Theranostic dendrimers for MRI

MRI is a widely used clinical diagnostic method. Three-dimensional images with high resolution, which reflect the state of water molecules, can be obtained noninvasively. Contrast agents that affect the relaxation time of protons of water are useful for MRI. Gadolinium (Gd (III)) ion-coordinated chelates are ideally suited for such contrast agents in T1-weighted MRI. However, current Gd (III)-based commercial agents have inefficient contrast enhancement capabilities and limited targeting activity to the sites of interest. Wiener et al. first synthesized a gadolinium-incorporated PAMAM dendrimer for MRI imaging (47). A single PAMAM dendrimer with many Gd chelates increased the relaxivity, which corresponds to the MR signal intensity. Because the relaxivity of the Gd (III) chelates affects the water exchange rate, the relaxivity of macromolecular Gd (III) chelates can be drastically increased upon slowing their molecular tumbling. High relaxivity and controlled pharmacokinetics of the contrast agents are critical for effective MRI. Many dendrimer-based macromolecular contrast agents have been developed by conjugating Gd (III) chelates to the periphery of the dendrimers (48). Like the PAMAM dendrimer, many nanoglobular (POSS core) dendrimers enable the conjugation of Gd (III) chelates. The pharmacokinetics and renal excretion characteristics of the nanoglobular contrast agents are the key parameters for controlling *in vivo* contrast enhancement in MRI (49).

Super paramagnetic iron oxide NPs (SPIO NPs) are a suitable contrast agent for T2-weighted MRI (50–52). The potential of the SPIO NPs in applications for drug/gene delivery has also been explored (53). A hybrid material comprising a pH-responsive PEGylated dendrimer (G2.5)/DOX conjugate with SPIO NPs was developed by Chang et al. (2011). The dendrimer was conjugated with DOX via a pH-cleavable hydrazone linkage and was successively complexed with SPIO NPs. The developed PEG-G2.5-DOX@SPIO NPs showed higher DOX release at pH 5.2 than pH 7.4. The authors explored the use of mPEG-G2.5-DOX@SPIO NPs as an MRI contrast agent for *in vivo* cancer detection. Tumors were visible as hyper-intense areas in T2-weighted MRI from 1 to 24 h after the injection, indicating that the complexes can be used as MR probes (54). The same authors integrated FA to achieve the target-specific delivery of DOX and SPIO NPs. The attachment of the targeting ligand to theranostic devices induced higher accumulation and retention at the tumor tissue compared with the nontargeted nanodevices (without FA) (55). Chang et al. (2013) have also illustrated the development of the SPIO NP for the delivery of TAX by following the same procedure. This dendrimer nanodevice associated with fluorescent imaging agent (Cy5.5) showed significant cellular uptake enhancement *in vitro* and enhanced the cytotoxicity of TAX toward tumor cells compared with the nontargeted conjugates. In PEG-3.5-TAX-Cy5.5@SPIO NPs-injected mice, the tumors were enhanced in T2-weighted MR images, and therapeutic effects were observed. The therapeutic delivery system integrated with imaging capability and a targeting ligand behaved successfully as both a diagnostic and a chemotherapeutic device (56).

Taratula et al. developed a synchronized system for specific delivery to cancer cells comprising siRNA and MRI contrast agents (SPIO) using dendrimers. SPIO NPs of 5-nm diameter were complexed with siRNA and a G5 PPI dendrimer. The formulated siRNA complexes were further modified with PEG bearing LHRH peptide as a targeting agent to provide a tumor-specific targeting moiety and inhibit nonspecific interactions. The dendrimer/siRNA/SPIO hybrid NPs bearing PEG-LHRH enhanced internalization into cancer cells and increased the efficiency of the targeted gene suppression *in vitro*. Moreover, the dendrimer nanodevice could be used to co-deliver the anticancer drug cisplatin, which exhibited enhanced *in vivo* antitumor activity (57).

Yoon et al. established an effective theranostic platform containing DPs for PDT and SPIO NPs for MRI. Multifunctional hollow polymeric nanocapsules (NCs) have been fabricated using the layer-by-layer (LbL) approach with negatively charged DP and positively charged poly(allylamines) and PEG-b-polylysine from an SPIO NP-embedded polystyrene NP core (Figure 3). The porphyrin unit in the core of the DP showed strong red fluorescence emission, and the SPIO NPs produced a strong MRI signal. Because the DP in the shells of the NCs works as a photosensitizer, the NCs also exhibited photocytotoxicity. Thus, the SPIO NP and DP hybrid material will have diverse applications in cancer therapy and diagnosis (58). In this NP, porphyrin, which is the active site in PDT, was isolated in the dendrimer, which could work effectively after the hybridization.

Theranostic dendrimers for CT imaging

X-ray CT is also a powerful diagnostic imaging technique with good cost-effectiveness for use in clinics (59, 60). Contrast agents are useful for CT imaging of specific tissues and organs. Currently, iodinated compounds are used as contrast agents in clinical applications. However, iodinated compounds present the general problems of short circulation time, potential renal toxicity, and lack of targeting specificity. Moreover, X-ray CT is a less sensitive imaging technique than MRI, and high concentrations of the iodine compounds are required. It has been reported that iodinated compounds can be integrated within polymer NPs (61). The iodinated agents have been conjugated to dendrimers to improve their biocompatibility and prolong their blood circulation times (62). Fu et al. reported a dendrimer-based CT imaging probe, where the dendrimer was made of a PEG core conjugated with reactive tri-iodo phthalimide moieties (63). Because Bi_2S_3 nanomaterials can attenuate X-ray penetration much more effectively than iodinated compounds at the same molar concentration of the active element (Bi versus I), dendrimer-stabilized Bi_2S_3 NPs are applicable to CT imaging. This nanodevice may be applicable as a versatile platform for therapy and targeted CT imaging in different biological systems (64).

Recently, gold (Au) NPs have been investigated as potent contrast agents for CT imaging (65). The Au nanomaterials have been entrapped by, or stabilized with, various kinds of dendrimers and used for CT imaging (66). For example, G5 PAMAM dendrimers modified with PEG have been used for the development of dendrimer-entrapped Au NPs (DENPs) (67, 68). Liu et al. reported a facile approach to fabricating PAMAM dendrimer-stabilized gold NPs (Au DSNPs) attached with FA. This nanodevice showed selective targeting and enhancement of imaging signaling in

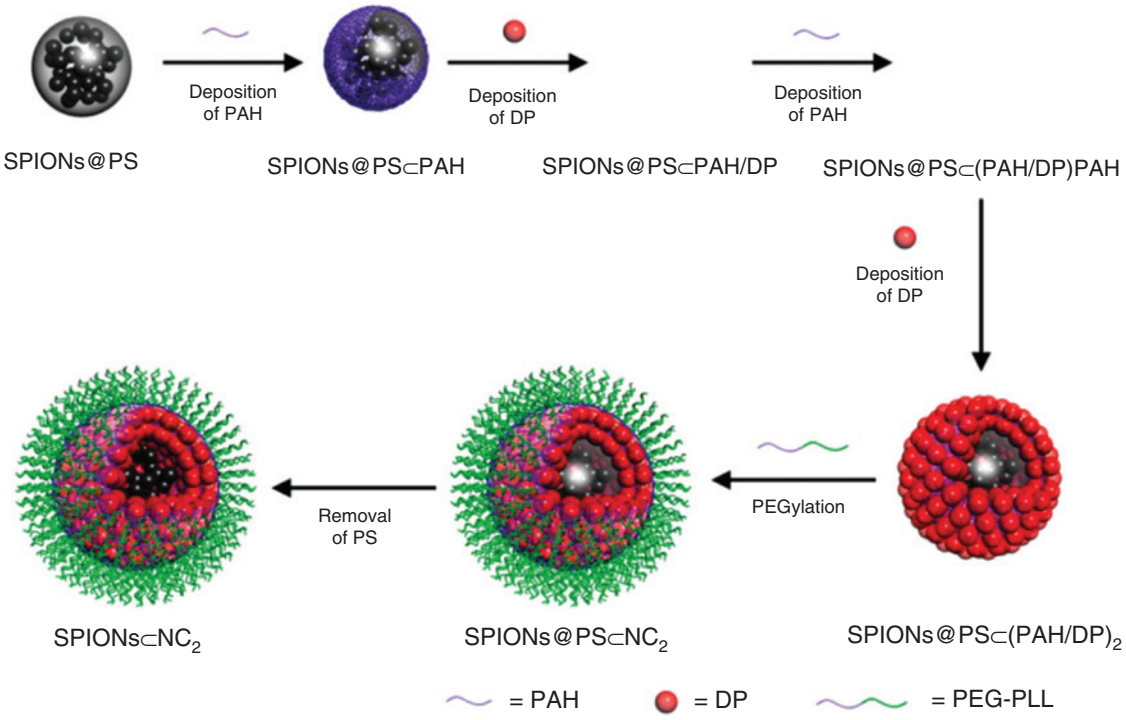

Figure 3: Preparation procedures for the fabrication of multifunctional hollow polymeric NCs made from SPIO NP and DP via the LbL method. Reproduced from Ref. (58). Copyright ©2014, American Chemical Society.

the KB cells of an induced mouse tumor model (Figure 4) (69). The same authors also illustrated the development of lactobionic acid (LA)-modified Au DENPs for *in vivo* CT imaging of targeted human hepatocellular carcinoma. The multifunctional Au DENPs had good stability at different pH and temperatures. Au DENPs were nontoxic to normal cells, but were cytotoxic to the targeted hepatocellular carcinoma cells (70). Kono and coworkers have developed Au NP-loaded PEGylated PAMAM dendrimers. The nanodevice showed both photocytotoxicity and CT imaging capability (71–73). Thus, Au DENPs and Au DSNPs are potent theranostic agents for PTT and CT imaging (73).

The Au DENPs covalently linked with α-TOS as a platform for targeted cancer CT imaging as well as for therapy. α-TOS affects tumor cells selectively, induces apoptosis in various types of cancer cells, and inhibits the cell cycle by disrupting the autocrine signaling pathways necessary for tumor growth. Because poor water solubility limited its application to cancer chemotherapy, the loading of α-TOS to carrier is indispensable. In a study by Zhu et al., FI, PEGylated α-TOS, and PEGylated FA were conjugated to a G5 amine-terminated PAMAM dendrimer, which was used as a template for Au DENP preparation. The FA modification of the Au DENPs enabled efficient targeting of cancer cells by the nanodevice, and effectively targeted CT imaging of the cancer cells *in vitro* and the xenografted tumor model *in vivo*. The X-ray attenuation of the NP was higher than that of the conventionally used iodine-based CT contrast agents, Omnipaque. The CT signal became faded, indicating that the dendrimer devices cleared out through metabolism. The property is very important for clinical applications. It is likely that the clearance of the NP resulted from the precise controllable structure of dendrimers. The developed Au-α-TOS-FA DENPs exhibited targeted cancer cell inhibition and antitumor effects in the mouse model (74).

Theranostic dendrimers for PET and SPECT imaging

Nuclear medicine imaging provides extremely high sensitivity and is applicable to whole-body quantitative analysis (75–77). PET and SPECT are nuclear imaging techniques that are capable of providing tomographic and quantitative functional information inside a living subject (78, 79). Radiolabeled compounds are used in these imaging techniques. These nuclear imaging probes have no biological counterparts. Dendrimer nanodevices have been used in PET and SPECT studies (77, 80). The dendrimer scaffold conjugated with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) can be

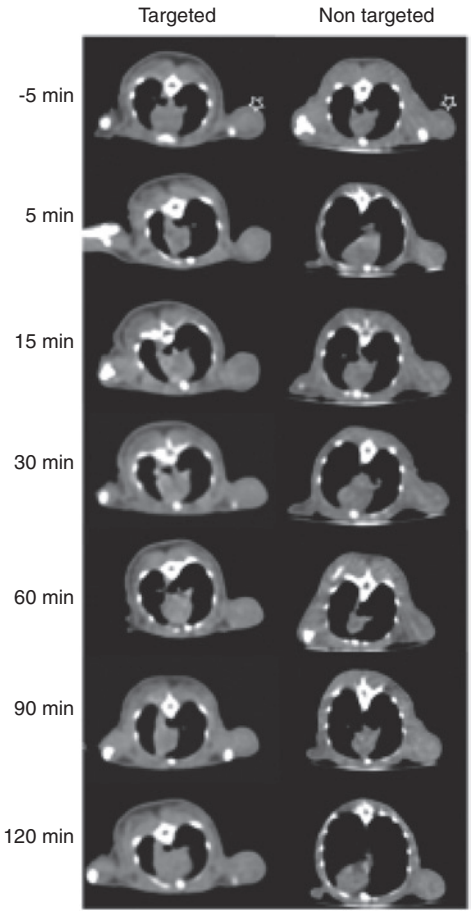

Figure 4: CT images of tumors before and after intravenous injection of DSNP with (targeted, left) or without FA (nontargeted, right). The stars indicate the tumor area. Reproduced from Ref. (69). Copyright ©2013, WILEYVCH Verlag GmbH & Co. KGaA, Weinheim.

labeled with many different radioisotopes, including ^{68}Ga, ^{64}Cu, and ^{86}Y for PET imaging (81, 82). The biological half-life of the dendrimer plays a key role in determining the proper radioisotope for a specific application. For instance, a low-generation (G0-G3) dendrimer that is rapidly cleared by the kidneys should be labeled with a short-lived radioisotope, such as ^{68}Ga or ^{64}Cu. In contrast, a high-generation dendrimer (>G7) with a long biological half-life should be labeled with a long-lived radioisotope, such as ^{111}In or ^{177}Lu. In the latter cases, the *in vivo* stability of the metal chelate moiety must be considered (83). Ideally, it is necessary to localize a sufficient concentration of the radioisotope to deliver a cytotoxic dose of radiation, as well as to remove it rapidly from the blood stream and other normal organs or tissues to minimize radiation damage. Several radionuclides are currently used for the treatment of malignancies.

PEG-block-dendron telodendrimers, which can assemble to form micelles, are promising nanocarriers for imaging-guided drug delivery, and have been developed by using a multifunctional telodendrimer micelle. Fluorophores and radionuclides can be easily labeled via covalent conjugation to telodendrimers through a lysine side chain. Xiao et al. covalently modified the telodendrimer with ^{125}I and loaded ^{14}C-TAX into the nanomicelles in a separate methodology. The pharmacokinetics and the biodistribution of the telodendrimer-based micelles were investigated using micro SPECT/CT imaging and liquid scintillation counting, respectively. ^{125}I-labeling nanomicelles were highly efficient in tumor targeting for SPECT/CT imaging, which indicates the nanomicelles derived nanoplatform as potential cancer imaging and diagnosis. Also, the biodistribution study of ^{14}C-PTX nanomicelles showed prolong systemic circulation in blood and improved uptake in the tumor mass. The results indicated that nanomicelle-formulated TAX showed efficient therapeutic activities and tumor-imaging properties (84).

PAMAM dendrimer-coated adenovirus vectors carrying the hNIS gene (theranostic sodium iodide symporter gene) have been developed by Grünwald et al. Significant reduction of hepatic accumulation and liver toxicity was observed after intravenous injection of dendrimer-coated adenovirus vectors carrying the hNIS gene (dcAd5-CMV/NIS). However, the transduction efficiency and accumulation of dcAd5-CMV/NIS at the tumor tissue increased. This delivery system allows imaging and radiovirotherapy of nonthyroidal cancers. Synergies between oncolytic virotherapy and NIS-mediated radionuclide therapy were also demonstrated (85). To further improve the targeting activity, the same authors also prepared a PAMAM dendrimer containing an epidermal growth factor receptor (EGFR)-specific ligand peptide (GE11), with which the adenovirus vectors carrying the hNIS gene were coated. High accumulation at the tumor tissues was observed, and the EGFR specificity was confirmed by decreased tumoral accumulation after pretreatment with the EGFR-specific antibody cetuximab (Figure 5). Additional treatment with a therapeutic dose of ^{131}I induced an efficient antitumor effect after the systemic application of dendrimer-coated adenoviruses (86).

Conclusion

Dendrimers have been widely used as nanovehicles for drug delivery or as nanodevices for imaging for some time.

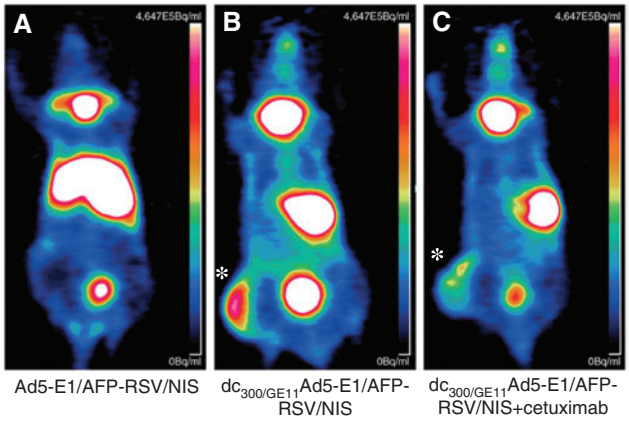

Figure 5: ^{124}I-PET images after intravenous injection of the uncoated virus vector (Ad5-E1/AFP-RSV/NIS) (A) and the adenovirus coated with PAMAM-G2-PEG-GE11 (dc300/GE11Ad5-E1/AFP-RSV/NIS) (B,C). By pretreatment with the monoclonal anti-EGFR antibody cetuximab, accumulation at tumor was significantly reduced. The stars indicate the tumor area. Reproduced from Ref. (85). Copyright ©2013, Rights Managed by Nature Publishing Group.

It is common practice to integrate drug- or gene-loaded dendrimers with FIs for *in vitro* fluorescence imaging. Because other diagnostics techniques, including MRI, CT, PET, and SPECT, are suitable for *in vivo* applications, research into theranostic dendrimers has increased. In this review, we discussed recent developments in various dendrimer-based theranostic nanomedicines and their biological impacts, which are summarized in Table 1. The literature indicates that most studies have focused on the development of diagnostic nanodevices rather than on theranostic ones. Theranostic agents for preclinical investigations using MRI, CT, and nuclear medicine imaging are still being developed. Owing to their multifunctionality, dendrimers are able to receive therapeutic agents, imaging agents, and targeting ligands in a single molecule. The inner space is available to retain molecules through electrostatic interactions, for example, and the outer surface is also available to conjugate to and/or complex with molecules, suggesting that dendrimers are an ideal nanoplatform for theranostics.

Expert opinion and outlook

Since the invention of dendrimers, they have evolved constantly and are usually found in therapeutic or diagnostic applications. However, their evolution in those fields has been independent. In the last 10 years, dendrimers have been used in several theranostic applications. The most interesting dendrimers integrate a therapeutic drug, an imaging agent, and a targeting ligand, constituting a single nanoformulation device. Preclinical studies have mostly focused on a variety of dendrimer NPs for use in imaging in cancer and other diseases. Many dendrimer-based diagnostic devices are currently at an early stage of development and may undergo preclinical trials for applications in various disease therapies. The development of theranostic dendrimer agents has mostly been based on fluorescence imaging and MRI applications. However, a very limited number of theranostic devices have been reported for CT or nuclear medical imaging, and there is great scope for future innovation in these fields.

Despite several advantages of dendrimers as a theranostic material, they still have problems regarding synthesis, cost-effectiveness, and *in vivo* safety for the practical applications. Although the dendrimer itself is monodispersed, the multifunctional dendrimer nanodevices possibly lose the dendrimers homogeneity during multistep modification. Thus, quantitative reactions are necessary for preparation of multifunctional dendrimers. Besides, synthesis of the dendrimer takes many costs. Although dendrimers of lower generations are not expensive, these dendrimers are easily excreted through the renal route. The control of *in vivo* fate remains a challenge. PEGylation and POSS core of the dendrimer are useful approaches for enlarging the dendrimer size and elongating blood circulation. Because dendrimers are not classical compounds, the *in vivo* safety is somehow unclear. The ongoing clinical trials will pave the way for clinical applications of other dendrimer nanodevices.

Highlights

1. The multifunctional properties of dendrimers allow them to combine imaging agents, drugs, and targeting ligands, making them suitable for therapy as well as diagnosis in biomedical applications.
2. Theranostic dendrimers for fluorescence imaging are prepared by adding an fluorescent dye to the drug- and/or gene-loaded dendrimer NP.
3. Dendrimers attached to gadolinium (Gd (III)) chelates have higher relaxivities in MRI, and the drug- and/or gene-loaded dendrimers bearing Gd (III) chelates are useful in theranostic applications.
4. Au NP-loaded dendrimers can be used in theranostic applications involving CT imaging.

Table 1: Summary of theranostic dendrimers and their application.

Sl. no.	Dendrimer type	Therapeutic agent	Diagnostic agent	Imagining	Disease target	Refs
1	G3 PAMAM dendrimer	MTX	FI	Fluorescence imaging	*In vitro* cytotoxicity in the FR-expressing KB cell line	(30)
2	G5 PAMAM dendrimer	MTX	FI	Fluorescence imaging	Efficiently binding with KB cells on flow cytometry experiment	(31)
3	G5 PAMAM dendrimer	TAX	Cy5	Fluorescence imaging	microtubules	(32)
4	G5 dendrimer with RGD	DOX	FI	Fluorescence imaging	Glioblastoma U87MG cancer cells	(33)
5	PAPAM dendrimer (AG nanogel formulation, $CaCl_2$ used as cross linker of the dendrimer)	DOX	FI	Fluorescence imaging	CAL-72 cells (a human osteosarcomacell line)	(34)
6	PAMAM dendrimer with LHRH peptide	TAX	Cy5.5	Fluorescence imaging	High antitumor therapeutic activity with low adverse side effects and high accumulation at the tumor	(38)
7	G2 cationic carbosilane dendrimer	siRNA	FI	Fluorescence imaging	Transfected siRNA to HIV-infected human primary astrocytes	(39)
8	G4 PPI with LHRH peptide	Pcs	Pcs	Fluorescence imaging	*In vitro* SKOV-3 human ovariancarcinoma, MCF-7 breast and PC-3 prostate cancer cell lines, and *in vivo* human ovarian carcinoma xenografts model	(44)
9	G5 PPI	Naphthalocyanine	Naphthalocyanine or SiNc	Fluorescence imaging	Combinatorial phototherapeutic effects to drug-resistant ovarian cancer *in vitro* and *in vivo*	(45)
10	PAMAM dendrimer	OligoDNA	FI	Fluorescence imaging	Breast cancer cells (MCF-7 and MDA-MB-435) and liver cancer HepG2 cell line	(47)
11	PEGylated G2.5 dendrimer	DOX	SPIO NPs	MRI	*In vivo* cancer detection in mice model	(54)
12	PEGylated PAMAM dendrimer G3.5	DOX	SPIO NPs	MRI	*In vivo* tumor tissue	(55)
13	mPEG-G3.5 PAMAM dendrimer	TAX	SPIO NPs	MRI	ICR male mice inoculated with H22 cells induced solid subcutaneous tumor	(56)
14	DPs	Porphyrin unit in dendrimer	SPIO NPs/ porphyrine core	MRI and fluorescence imaging	HeLa cell line	(58)
15	G4 PAMAM dendrimers glycidol hydroxyl-terminated	NA	Bi	CT	Rabbit and mouse CT image	(64)
16	PAMAM dendrimers	Au NPs	Au NPs	CT	*In vitro* and *in vivo* targeted CT imaging of human hepatocellular carcinoma	(70)
17	G5 PAMAM dendrimer	α-Tocopheryl succinate	FI	CT	FAR-overexpressing cancer cells *in vitro* and the xenografted tumor model *in vivo*	(74)
18	Telodendrimer micelle	TAX	^{125}I	SPECT/CT	Mouse xenograft model of ovarian cancer	(84)
19	G5 PAMAM dendrimer	hNIS gene	^{123}I	PET	^{123}I scintigraphy and NIS-mediated therapy in a liver cancer xenograft moue model	(85)
20	Cationic PAMAM dendrimer	Adenoviral NIS gene therapy	^{124}I	PET	Increased transduction efficiency in peripheral xenograft tumors	(86)

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List of abbreviations

AG	alginate nanogels
Au DENPs	dendrimer-entrapped gold nanoparticles
Au DSNPs	dendrimer-stabilized gold nanoparticles
Au NP	gold nanoparticle
CT	X-ray computed tomography
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DOX	doxorubicin
DP	dendrimer porphyrin
EGFR	epidermal growth factor receptor
FA	folic acid
FI	fluorescein isothiocyanate
FR	folic acid receptor
LbL	layer-by-layer
LHRH	luteinizing hormone-releasing hormone
MNPs	magnetic nanoparticles
MRI	magnetic resonance imaging
MTX	methotrexate
NC	nanocapsules
NIR	near-infrared
PAMAM	polyamidoamine
Pc	phthalocyanine
PDT	photodynamic therapy
PEG	polyethylene glycol
PET	positron emission tomography
POSS	polyhedral oligomeric silsesquioxane
PPI	poly(propyleneimine)
PTT	photothermal therapy
RGD	arginyl-glycyl-aspartic acid
ROS	reactive oxygen species
SiNc	silicon 2,3-naphthalocyanine bis(trihexylsilyloxide)
siRNA	small interfering RNA
SPECT	single photon emission computed tomography
SPIO NPs	super paramagnetic iron oxide nanoparticles
TAX	paclitaxel
α-TOS	α-tocopheryl succinate

References

1. McCarthy JR, Weissleder R. Multifunctional magnetic nanoparticles for targeted imaging and therapy. Adv Drug Deliv Rev 2008; 60: 1241–51.
2. Rawat M, Singh D, Saraf S, Saraf S. Nanocarriers: promising vehicle for bioactive drugs. Biol Pharm Bull 2006; 29: 1790–8.
3. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005; 4: 145–59.
4. Tomalia DA, Naylor AM, Goddard WA. Starburst dendrimers: molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter. Angew Chem Int Ed Engl 1990; 29: 138–75.
5. Chen Q, Li K, Wen S, Liu H, Peng C, Cai H, Shen M, Zhang G, Shia X. Targeted CT/MR dual mode imaging of tumors using multifunctional dendrimer-entrapped gold nanoparticles. Biomaterials 2013; 34: 5200–9.
6. Wen S, Li K, Cai H, Chen Q, Shen M, Huang Y, Peng C, Hou W, Zhu M, Zhang G, Shia S. Multifunctional dendrimer entrapped gold nanoparticles for dual mode CT/MR imaging applications. Biomaterials 2013; 34: 1570–80.
7. Zhu J, Shi X. Dendrimer-based nanodevices for targeted drug delivery applications. J Mater Chem 2013; 1: 4199–211.
8. Sk UH, Dixit D, Sen E. Comparative study of microtubule inhibitors – estramustine and natural podophyllotoxin conjugated PAMAM dendrimer on glioma cell proliferation. Eur J Med Chem 2013; 68: 47–57.
9. Kojima C, Suehiro T, Watanabe K, Ogawa M, Fukuhara A, Nishisaka E, Harada A, Kono K, Inui T, Magata Y. Doxorubicin-conjugated dendrimer/collagen hybrid gels for metastasis-associated drug delivery systems. Acta Biomater 2013; 9: 5673–80.
10. Bulte JW, Douglas T, Witwer B, Zhang SC, Strable E, Lewis BK, Zywicke H, Miller B, van Gelderen P, Moskowitz BM, Duncan ID, Frank JA. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nat Biotechnol 2001; 19: 1141–7.
11. Soundararajan A, Bao A, Phillips WT, Perez R, III Goins B A. [(^{186})Re]liposomal doxorubicin (Doxil): in vitro stability, pharmacokinetics, imaging and biodistribution in a head and neck squamous cell carcinoma xenograft model. Nucl Med Biol 2009; 36: 515–24.
12. Lim J, Lo ST, Hill S, Pavan GM, Sun X, Simanek EE. Antitumor activity and molecular dynamics simulations of paclitaxelladen triazine dendrimers. Mol Pharm 2012; 9: 404–12.
13. Lo ST, Stern S, Clogston JD, Zheng J, Adiseshaiah PP, Dobrovolskaia M, Lim J, Patri A K, Sun X, Simanek EE. Biological assessment of triazine dendrimer: toxicological profiles, solution behavior, biodistribution, drug release and efficacy in a PEGylated, paclitaxel construct. Mol Pharm 2010; 7: 993–1006.
14. Lee IH, An S, Yu MK, Kwon HK, Im SH, Jon S. Targeted chemoimmunotherapy using drug-loaded aptamer-dendrimer bioconjugates. J Control Release 2011; 155: 435–41.
15. Liu WM, Xue YN, He WT, Zhuo RX, Huang SW. Dendrimer modified magnetic iron oxide nanoparticle/DNA/PEI ternary complexes: a novel strategy for magnetofection. J Control Release 2011; 152: e159–60.
16. Xu R, Wang Y, Wang X, Jeong EK, Parker DL, Lu ZR. In vivo evaluation of a PAMAM-cystamine-(Gd-DO3A) conjugate as a biodegradable macromolecular MRI contrast agent. Exp Biol Med (Maywood) 2007; 232: 1081–9.
17. Schlick KH, Udelhoven RA, Strohniever GC, Cloninger MJ. Binding of mannose-functionalized dendrimers with pea (Pisum sativum) lectin. Mol Pharm 2005; 2: 295–301.

18. Yang W,Cheng Y, Xu T, Wang X, Wen LP. Targeting cancer cells with biotin dendrimer conjugates. Eur J Med Chem 2009; 44: 862–8.
19. Xu L, Yeudall WA, Yang H. Dendrimer-based RNA interference delivery for cancer therapy. In: Scholz C, Kressler J, editors. Tailored Polymer Architectures for Pharmaceutical and Biomedical Applications. Washington, DC: American Chemical Society, 2013: 197–213.
20. McCarthy TD, Karellas P, Henderson SA, Giannis M, O'Keefe DF, Heery G, Paull JRA, Matthews BR, Holan G. Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. Mol Pharm 2005; 2: 312–8.
21. Kannan RM, Nance E, Kannan S, Tomalia DA. Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. J Intern Med 2014; 276: 579–617.
22. Xu L, Zhang H, Wu Y. Dendrimer advances for the central nervous system delivery of therapeutics. ACS Chem Neurosci 2014; 5: 2–13.
23. Fahmy TM, Fong PM, Park J, Constable T, Saltzman WM. Nanosystems for simultaneous imaging and drug delivery to T cells. AAPS J 2007; 9: E171–80.
24. Nanjwade BK, Bechra HM, Derkar GK, Manvi FV, Nanjwade VK. Dendrimers: emerging polymers for drug-delivery systems. Eur J Pharm Sci 2009; 38: 185–96.
25. Niederhafner P, Reinis M, Sebestik J, Jezek J. Glycopeptide dendrimers, part III: a review. Use of glycopeptides dendrimers in immunotherapy and diagnosis of cancer and viral diseases. J Pept Sci 2008; 14: 556–87.
26. Mintzer MA, Grinstaff MW. Biomedical applications of dendrimers: a tutorial. Chem Soc Rev 2011; 40: 173–90.
27. Giepmans BN, Adams SR, Ellisman MH, Tsien RY. The fluorescent toolbox for assessing protein location and function. Science 2006; 312: 217–24.
28. Lakowicz JR. Principles of fluorescence spectroscopy. New York: Springer, 2006: 954.
29. Majoros IJ, Thomas TP, Mehta CB, Baker JR. Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy. J Med Chem 2005; 48: 5892–9.
30. Zhang Y, Thomas TP, Lee KH, Li M, Zong H, Desai AM, Kotlyar A, Huang B, Holl MM, Baker JR Jr. Polyvalent saccharide-functionalized generation 3 poly(amidoamine) dendrimer-methotrexate conjugate as a potential anticancer agent. Bioorg Med Chem 2011; 19: 2557–64.
31. Huang B, Kukowska-Latallo JF, Tang S, Zong H, Johnson KB, Desai A, Gordon CL, Leroueil PR, Baker JR Jr. The facile synthesis of multifunctional PAMAM dendrimer conjugates through copper-free click chemistry. Bioorg Med Chem Lett 2012; 22: 3152–6.
32. Cline EN, Li MH, Choi SK, Herbstman JF, Kaul N, Meyhöfer E, Skiniotis G, Baker JR, Larson RG, Walter NG. Paclitaxel-conjugated PAMAM dendrimers adversely affect microtubule structure through two independent modes of action. Biomacromolecules 2013; 14: 654–64.
33. He X, Alves CS, Oliveira N, Rodrigues J, Zhu J, Bányai I, Tomás H, Shi X. RGD peptide-modified multifunctional dendrimer platform for drug encapsulation and targeted inhibition of cancer cells. Colloids Surf B Biointerfaces 2015; 125: 82–9.
34. Gonçalves M, Maciel D, Capelo D, Xiao S, Sun W, Shi X, Rodrigues J, Tomás H, Li Y. Dendrimer-assisted formation of fluorescent nanogels for drug delivery and intracellular imaging. Biomacromolecules 2014; 15: 492–9.
35. Dharap SS, Qiu B, Williams GC, Sinko P, Stein S, Minko T, Molecular targeting of drug delivery systems to ovarian cancer by BH3 and LHRH peptides, J Control Release 2003; 91: 61–73.
36. Dharap SS, Minko T. Targeted proapoptotic LHRH-BH3 peptide, Pharm Res 2003; 20: 889–96.
37. Minko T, Khandare J, Jayant S. In: Matyjaszewski K, Gnanou Y, Leibler L, editors. Macromolecular engineering: from precise macromolecular synthesis to macroscopic material properties and application. Weinheim: Wiley-VCH Verlag GmbH & Co., vol. 4, 2007: 2541–95.
38. Saad M, Garbuzenko OB, Ber E, Chandna P, Khandare JJ, Pozharov VP, Minko T. Receptor targeted polymers, dendrimers, liposomes: which nanocarrier is the most efficient for tumor-specific treatment and imaging? J Control Release 2008; 130: 107–14.
39. Serramía MJ, Álvarez S, Fuentes-Paniagua E, Clemente MI, Sánchez-Nieves J, Gómez R, de la Mata J, Muñoz-Fernández MÁ. In vivo delivery of siRNA to the brain by carbosilane dendrimer. J Control Release 2015; 200: 60–70.
40. Pan B, Cui D, Sheng Y, Ozkan C, Gao F, He R, Li Q, Xu P, Huang T. Dendrimer-modified magnetic nanoparticles enhance efficiency of gene delivery system. Cancer Res 2007; 67: 8156–63.
41. Buccella D, Horowitz JA, Lippard SJ. Understanding zinc quantification with existing and advanced ditopic fluorescent Zinpyr sensors. J Am Chem Soc 2011; 133: 4101–14.
42. Jiang DL, Aida T. Morphology-dependent photochemical events in aryl ether dendrimer porphyrins: cooperation of dendron subunits for singlet energy transduction. J Am Chem Soc 1998; 120: 10895–901.
43. Nishiyama N, Stapert HR, Zhang GD, Takasu D, Jiang DL, Nagano T, Aida T, Kataoka K. Light-harvesting ionic dendrimer porphyrins as new photosensitizers for photodynamic therapy. Bioconjug Chem 2003; 14: 58–66.
44. Taratula O, Schumann C, Naleway MA, Pang AJ, Chon KJ, Taratula O. A multifunctional theranostic platform based on phthalocyanine-loaded dendrimer for image-guided drug delivery and photodynamic therapy. Mol Pharm 2013; 10: 3946–58.
45. Taratula O, Schumann C, Duong T, Taylor KL, Taratula O. Dendrimer-encapsulated naphthalocyanine as a single agent-based theranostic nanoplatform for near-infrared fluorescence imaging and combinatorial anticancer phototherapy. Nanoscale 2015; 7: 3888–902.
46. Shao NM, Su YZ, Hu JJ, Zhang JH, Zhang HF, Cheng YY. Comparison of generation 3 polyamidoamine dendrimer and generation 4 polypropylenimine dendrimer on drug loading, complex structure, release behavior, and cytotoxicity. Int J Nanomed 2011; 6: 3361–72.
47. Wiener EC, Brechbiel MW, Brothers H, Magin RL, Gansow OA, Tomalia DA, Lauterbur PC. Dendrimer-based metal-chelates – a new class of magnetic-resonance-imaging contrast agents. M Magn Reson Med 1994; 31: 1–8.
48. Longmire M, Choyke PL, Kobayashi H. Dendrimer-based contrast agents for molecular imaging. Curr Top Med Chem 2008; 8: 1180–6.
49. Xu R, Kaneshiro TL, Jeong EK, Parker DL, Lu ZR. Synthesis and evaluation of nanoglobule–cystamine–(Gd-DO3A), a biodegradable nanosized magnetic resonance contrast agent for dynamic contrast-enhanced magnetic resonance urography. Int J Nanomed 2010; 5: 707–13.

50. Thorek DLJ, Chen A, Czupryna J, Tsourkas A. Superparamagnetic iron oxide nanoparticle probes for molecular imaging. Ann Biomed Eng 2006; 34: 23–38.
51. Lee JH, Lee K, Moon SH, Lee Y, Park TG, Cheon J. All-in-one target-cell-specific magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery. Angew Chem Int Ed 2009; 48: 4174–9.
52. Lee H, Lee E, Kim DK, Jang NK, Jeong YY, Jon S. Antibiofouling polymer-coated superparamagnetic iron oxide nanoparticles as potential magnetic resonance contrast agents for in vivo cancer imaging. J Am Chem Soc 2006; 128: 7383–9.
53. Morishita N, Nakagami H, Morishita R, Takeda S, Mishima F, Terazono B, Nishijima S, Kaneda Y, Tanaka N. Magnetic nanoparticleswi th surface modification enhanced gene delivery of HVJ-E vector. Biochem Biophys Res Comm 2005; 334: 1121–6.
54. Chang Y, Meng X, Zhao Y, Li K, Zhao B, Zhu M, Li Y, Chen X, Wang J. Novel water-soluble and pH-responsive anticancer drug nanocarriers: doxorubicin-PAMAM dendrimer conjugates attached to superparamagnetic iron oxide nanoparticles (IONPs). J Colloid Interface Sci 2011; 363: 403–9.
55. Chang YL, Liu N, Chen L, Meng X L, Liu Y J, Li YP, Wang JY. Synthesis and characterization of DOX-conjugated dendrimer-modified magnetic iron oxide conjugates for magnetic resonance imaging, targeting, and drug delivery. J Mater Chem 2012; 22: 9594–601.
56. Chang Y, Li Y, Meng X, Liu N, Sun D, Liu H, Wang J. Dendrimer functionalized water soluble magnetic iron oxide conjugates as dual imaging probe for tumor targeting and drug delivery. Polym Chem 2013; 4: 789–94.
57. Taratula O, Garbuzenko O, Savla R, Wang YA, He H, Minko T. Multifunctional nanomedicine platform for cancer specific delivery of siRNA by superparamagnetic iron oxide nanoparticles-dendrimer complexes. Curr Drug Deliv 2011; 8: 59–69.
58. Yoon HJ, Lim TG, Kim JH, Cho YM, Kim YS, Chung US, Kim JH, Choi BW, Koh WG, Jang WD. Fabrication of multifunctional layer-by-layer nanocapsules toward the design of theragnostic nanoplatform. Biomacromolecules 2014; 15: 1382–9.
59. deKrafft KE, Xie Z, Cao G, Tran S, Ma L, Zhou OZ, Lin W. Iodinated nanoscale coordination polymers as potential contrast agents for computed tomography. Angew Chem Int Ed Engl 2009; 121: 10085–8.
60. Liu Y, Ai K, Liu J, Yuan Q, He Y, Lu L. A high-performance ytterbium-based nanoparticulate contrast agent for in vivo X-ray computed tomography imaging. Angew Chem Ed Engl 2012; 124: 1466–71.
61. Aviv H, Bartling S, Kieslling F, Margel S. Radiopaque iodinated copolymeric nanoparticles for X-ray imaging applications. Biomaterials 2009; 30: 5610–6.
62. Guo R, Wang H, Peng C, Shen M, Zheng L, Zhang G, Shi X. Enhanced X-ray attenuation property of dendrimer-entrapped gold nanoparticles complexed with diatrizoic acid. J Mater Chem 2011; 21: 5120–7.
63. Fu Y, Nitecki DE, Maltby D, Simon GH, Berejnoi K, Raatschen HJ, Yeh BM, Shames DM, Brasch RC. Dendritic iodinated contrast agents with PEG-cores for CT imaging: synthesis and preliminary characterization. Bioconjug Chem 2006; 17: 1043–56.
64. Fang Y, Peng C, Guo R, Zheng L, Qin J, Zhou B, Shen M, Lu X, Zhang G, Shi X. Dendrimer-stabilized bismuth sulfide nanoparticles: synthesis, characterization, and potential computed tomography imaging applications. Analyst 2013; 138: 3172.
65. Zhou B, Zheng L, Peng C, Li D, Li J, Wen S, Shen M, Zhang G, Shi X. Synthesis and characterization of PEGylated polyethylenimine-entrapped gold nanoparticles for blood pool and tumor CT imaging. ACS Appl Mater Interfaces 2014; 6: 17190–9.
66. Wang H, Zheng L, Peng C, Guo R, Shen M, Shi X, Zhang G. Computed tomography imaging of cancer cells using acetylated dendrimer-entrapped gold nanoparticles. Biomaterials 2011; 32: 2979–88.
67. Peng C, Zheng L, Chen Q, Shen M, Guo R, Wang H, Cao X, Zhang G, Shi X. PEGylated dendrimer-entrapped gold nanoparticles for in vivo blood pool and tumor imaging by computed tomography. Biomaterials 2012; 33: 1107–19.
68. Wang H, Zheng L, Guo R, Peng C, Shen M, Shi X, Zhang G. Dendrimer-entrapped gold nanoparticles as potential CT contrast agents for blood pool imaging. Nanoscale Res Lett 2012; 7: 190.
69. Liu H, Xu Y, Wen S, Chen Q, Zheng L, Shen M, Zhao J, Zhang G, Shi X. Targeted tumor computed tomography imaging using low-generation dendrimer-stabilized gold nanoparticles. Chem Eur J 2013; 19: 6409–16.
70. Liu H, Wang H, Xu Y, Guo R, Wen S, Huang Y, Liu W, Shen M, Zhao J, Zhang G, Shi X. Lactobionic acid-modified dendrimer-entrapped gold nanoparticles for targeted computed tomography imaging of human hepatocellular carcinoma. ACS Appl Mater Interfaces 2014; 6: 6944–53.
71. Umeda Y, Kojima C, Harada A, Horinaka H, Kono K. PEG-attached dendrimers encapsulating gold nanoparticles: growing gold nanoparticles in the dendrimers for improvement of their photothermal properties. Bioconj Chem 2010; 21: 1559–64.
72. Kojima C, Umeda Y, Ogawa M, Harada A, Magata Y, Kono K. X-ray computed tomography contrast agents prepared by seeded growth of gold nanoparticles in PEGylated dendrimer. Nanotechnology 2010; 21: 245104.
73. Kojima C, Cho SH, Higuchi E. Gold nanoparticle-loaded PEGylated dendrimers for theragnosis. Res Chem Intermed 2012; 38: 1279–89.
74. Zhu J, Zheng L, Wen S, Tang Y, Shen M, Zhang G, Shi X. Targeted cancer theranostics using alpha-tocopheryl succinate-conjugated multifunctional dendrimer-entrapped gold nanoparticles. Biomaterials 2014; 35: 7635–46.
75. O'Farrell AC, Shnyder SD, Marston G. Non-invasive molecular imaging for preclinical cancer therapeutic development. Br J Pharmacol 2013; 169: 719–35.
76. Kobayashi H, Longmire MR, Ogawa M, Choyke PL. Rational chemical design of the next generation of molecular imaging probes based on physics and biology: mixing modalities, colors and signals. Chem Soc Rev 2011; 40: 4626–48.
77. Kojima C, Niki Y, Ogawa M, Magata Y. Prolonged local retention of subcutaneously injected polymers monitored by noninvasive SPECT imaging. Int J Pharm 2014; 476: 164–8.
78. Phelps ME. PET: the merging of biology and imaging into molecular imaging. J Nucl Med 2000; 41: 661–81.
79. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Gene Dev 2003; 17: 545–80.
80. Matsuura N, Rowlands JA. Towards new functional nanostructures for medical imaging. Med Phys 2008; 35: 4474–87.
81. Notni J, Pohle K, Wester HJ. Comparative gallium-68 labeling of TRAP-, NOTA-, and DOTA-peptides: practical consequences for the future of gallium-68-PET. EJNMMI Res 2012; 2: 28.

82. Biddlecombe GB, Rogers BE, de Visser M, Parry JJ, de Jong M, Erion JL, Lewis JS. Molecular imaging of gastrin releasing peptide receptor-positive tumors in mice using ^{64}Cu- and ^{86}Y-DOTA-(Pro^{1}, Tyr^{4})-bombesin (1–14). Bioconjugate Chem 2007; 18: 724–30.
83. Lo ST, Kumar A, Hsieh JT, Sun X. Dendrimer nanoscaffolds for potential theranostics of prostate cancer with a focus on radiochemistry. Mol Pharm 2013; 10: 793–812.
84. Xiao W, Luo J, Jain T, Riggs JW, Tseng HP, Henderson PT, Cherry SR, Rowland D, Lam KS. Biodistribution and pharmacokinetics of a telodendrimer micellar paclitaxel nanoformulation in a mouse xenograft model of ovarian cancer. Int J Nanomedicine 2012; 7: 1587–97.
85. Grünwald GK, Vetter A, Klutz K, Willhauck MJ, Schwenk N, Senekowitsch-Schmidtke R, Schwaiger M, Zach C, Wagner E, Göke B, Holm PS, Ogris M, Spitzweg C. Systemic image-guided liver cancer radiovirotherapy using dendrimer-coated adenovirus encoding the sodium iodide symporter as theranostic gene. J Nucl Med 2013; 54: 1450–7.
86. Grünwald GK, Vetter A, Klutz K, Willhauck MJ, Schwenk N, Senekowitsch-Schmidtke R, Schwaiger M, Zach C, Wagner E, Göke B, Holm PS, Ogris M, Spitzweg C. EGFR-targeted adenovirus dendrimer coating for improved systemic delivery of the theranostic NIS gene. Mol Ther Nucleic Acids 2013; 2: e131.