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Dendrimer Nanoscaffolds for Potential Theranostics of Prostate Cancer with a Focus on Radiochemistry

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Abstract

Dendrimers are a class of structurally defined macromolecules featured with a central core, a low-density interior formed by repetitive branching units, and a high-density exterior terminated with surface functional groups. In contrast to their polymeric counterparts, dendrimers are nano-sized and symmetrically shaped, which can be reproducibly synthesized in a large scale with monodispersity. These unique features have made dendrimers of increasing interest for drug delivery and other biomedical applications as a nanoscaffold system. Intended to address the potential use of dendrimers for the development of theranostic agents, which combines therapeutics and diagnostics in a single entity for personalized medicine, this review focuses on the reported methodologies of using dendrimer nanoscaffolds for targeted imaging and therapy of prostate cancer. Of particular interest, relevant chemistry strategies are discussed due to their important roles in the design and synthesis of diagnostic and therapeutic dendrimer-based nanoconjugates and potential theranostic agents, targeted or non-targeted. Given the developing status of nanoscaffolded theranostics, major challenges and potential hurdles are discussed along with the examples representing current advances.

Keywords

Dendrimer; Theranostics; Prostate Cancer; Molecular Imaging; Drug Delivery

INTRODUCTION

Current therapeutic strategies often target one disease with the same regimen in different individual patients. In general, the delivery schedule and treatment dosage of each agent is given based on limited number of parameters of the disease such as stage, symptom, and physical condition of patient. Overwhelming literature reports have indicated that distinct different genetic makeup exists among patients even diagnosed with the same disease. For example, the success of Herceptin[®] attests to the viability of personalized therapies for cancer. Herceptin[®] is a monoclonal antibody that binds to the HER2/neu receptor that is over-expressed on the cell surface of approximately 25% of breast cancers.¹ Only HER2 positive cancers are responsive to treatment. The need for more refined, tailored treatment

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was also made clear by Iressa[®] for lung cancer treatment. While highly effective in 10% of lung cancer patients, it failed to enhance survival in the other treated patients. Recently it has been discovered that patients who respond to Iressa[®] have a somatic mutation in the tyrosine kinase domain of epidermal growth factor receptor (EGFR).²⁻⁵ These examples stress the need for a detailed molecular diagnosis and correspondingly a more tailored therapy regime.⁶ The concept of personalized medicine becomes more apparent because molecular medicine has clearly identified key genetic defects associated with many diseases. In order to formulate specific regimen for each patient, noninvasive molecular imaging techniques are needed in addition to genetic profiling in order to better define the location and extent of disease and to better assess the tumor response to drugs in a real-time manner.

Capable of providing highly specific information in the intact organism with respect to structural and functional phenotypes, molecular imaging has evolved to become an indispensable tool in biomedical research. The potential of molecular imaging has been well recognized for enhancing basic biological knowledge, better understanding molecular mechanisms of disease for early and accurate diagnosis, facilitating drug discovery and validation, and improving prediction and assessment of the response of a disease to various kinds of therapy. Most molecular imaging procedures are enabled by imaging probes, which usually consist of two components: a reporter group for detectable signal generation and a targeting moiety for localization of molecular events. Common reporter groups include fluorescent molecules for optical imaging, T_1 and T_2 contrast agents for magnetic resonance imaging (MRI), microbubbles for ultrasound imaging (US), radioisotopes for positron emission tomography (PET) and single photon emission computed tomography (SPECT). Targeting molecules are chosen based on the specific targets of interest. Usually, they can be small organic molecules, peptides, oligonucleotides, macromolecules such as antibodies, or activatable enzyme substrates.

The recently emerged concept termed as “*Theranostics*”, which integrates *therapeutic* and *diagnostic* agents into a single entity, is expected to play an important role in the personalized therapy.⁷ The central hypothesis of this concept is that the integration of a molecular imaging component would enable the desired noninvasive imaging of the *in vivo* status of the molecular target during the personalized treatment, to which the therapeutic agent is intended to be delivered. Of course, the personalized treatment must be administered after the final molecular diagnosis of the disease status, which can be performed by either the theranostic agent itself but void of the therapeutic component or other diagnostic techniques. The former is exemplified by a nuclear medicine practice that can date back to the 1940s, which uses iodine-123 (a γ -emitter) for diagnosis and then iodine-131 that emits both γ -rays and β -particles for imaging and radiotherapy of differentiated thyroid therapy.⁸ The latter would be a typical clinical practice, in which molecular diagnosis is firstly performed to identify and stratify patients based on the molecular signatures of their disease and then a corresponding theranostic agent is administered for both treatment and imaging-enabled noninvasive monitoring of the disease responses.

Not intended for an exhaustive coverage of literature on the design and potential application of theranostic agents, this review focuses on the recent advances of using dendrimer nanoscaffolds for the development of diagnostics or therapeutics and potential theranostic agents for prostate cancer with the emphasis on the radioisotope-enable approaches. Comprehensive reviews on the design of dendrimer nanoscaffolds for drug delivery can be found elsewhere.⁹⁻¹³ According to the American Cancer Society’s 2012 Facts and Figures, prostate cancer has been the most commonly diagnosed cancer in males and consistently among the leading causes of cancer-related deaths of men in United States. Although surgery can effectively control the primary prostate cancer, for metastatic disease, androgen

deprivation becomes a gold standard of therapy that only delays the onset of castration resistant prostate cancer (CRPC), which contributes to the majority of prostate cancer mortality. It is almost certain that the relapse of CRPC is inevitable for patients who are under hormonal therapy. With respect to current regimens available for the recurrent CRPC patients, some have shown therapeutic efficacy despite that there are many undesirable side effects (e.g. bone marrow suppression, alopecia, and sloughing of the epithelial cells in gut).¹⁴ Although tremendous efforts have been seen on the development of novel diagnostics and therapeutics towards the eventual cure of prostate cancer, few effective agents have been reported.^{15, 16}

Dendrimers are a class of highly branched and symmetrical macromolecular constructs, which consist of repeating dendrons extending outward from a central core.¹⁷⁻¹⁹ Compared to linear polymers, dendrimers can be synthesized reproducibly with low polydispersity, which is a highly desirable feature for drug delivery vehicles. The synthesis of dendrimers can be performed in a controlled manner, yielding products with predictable sizes termed as “generation”. Because of this controlled feature, different generations of a dendrimer system can be exploited to fulfill desired *in vivo* pharmacokinetic requirements for a wide range of biomedical applications (Figure 1). In addition, as the dendrimer core branches out in the synthesis of each generation, the number of peripheral groups increases exponentially, which results in a shielded interior that can be used to load drugs for delivery. Obviously, the peripheral functional groups, often in the form of amine or carboxylate, can be used to conjugate with functional molecules in a multivalent format for imaging signal amplification and therapeutic efficacy enhancement. Given the unique structural features, undoubtedly, dendrimers can be used as nanoscaffolds to develop theranostic agents for oncologic and nononcological applications.

In the past decades, the developmental efforts of theranostics have been focused on the use of currently available nanotechnologies and nanomaterials.^{7, 20-23} In this review, we discuss potential methods in the development of dendrimer nanoscaffolds for theranostic agents of prostate cancer.

DENDRIMER NANOSCAFFOLDS

Nanotechnologies have been extensively explored for more effective cancer diagnosis and therapy in the past decades. Of the basic requirements for cancer therapeutic or diagnostic agents, the pharmacokinetic properties of potential nano-sized platforms are critically important given the fact that they must be able to permeate the vascular wall, reach their biological targets, and retain there at a sufficiently high concentration for effective therapy or imaging. Further if the oncologic targets are intracellularly located, they must be able to cross the cell membrane. Engineered nanoparticles with the size range of several nanometers to a couple of hundred nanometers in diameter can be potentially designed to have desired pharmacokinetics, multiple binding capacities, and the ability to fit into an endocytotic vesicle through endocytosis or phagocytosis as a carrier or transporter of imaging reporters and/or therapeutic agents.²⁴ The reader is referred to other review articles²⁵⁻²⁷ for recent advances in nanoparticle-based molecular imaging research.

Among the nanoconstructs that have been reported for biomedical applications, dendrimers hold great promising in that they can be synthesized in a more controlled way with low polydispersity or monodispersity to achieve the desired *in vivo* pharmacokinetics for different applications by simply varying the generation (Figure 1). However, the tunable properties, such as passive and active tumor targeting, vary with different dendrimer systems. It is noteworthy that currently no general agreement has been reached on the size threshold of dendrimer systems that undergo the EPR tumor trapping mechanism. Like their

polymeric counterparts, dendrimers can provide hundreds or even thousands of sites if necessary for multi-presentation of various functional molecules to serve the purpose of interest, such as targeting ligands, imaging reporters, or therapeutic drugs. As discussed above, the major advantages of using dendrimers for cancer imaging or therapy include their more defined and readily tunable size and surface functionalities. Theoretically, the number of exterior functional groups branching out from the core increases exponentially generation-wise. Therefore, dendrimers can also be used as a nanocapsule system for drug loading (Figure 1). However, as the generation goes, the steric effects between the surface terminal groups and their spatial crowding would result in a higher generation dendrimer with less defined structure, which is not desirable for drug delivery. A dendrimer system, which is usually much smaller than its polymeric counterpart, exhibits rapid blood clearance and low accumulation in the reticuloendothelial system (RES) organs as compared to the *in vivo* behavior of other nanoconstructs. This provides compelling reasons to exploit dendrimers as biocompatible nanoscaffolds to carry imaging and/or therapeutic agents for biomedical applications. Indeed, specific tumor accumulation can be conveniently achieved by targeted nanoscaffolds as exemplified by a monoclonal antibody conjugated generation-4 poly(amidoamine) dendrimer (G4-PAMAM).²⁸ However, there is no guarantee that a dendrimer system would have an optimal *in vivo* pharmacokinetic profile. For a specific biological application, subjecting the dendrimer system to *in vitro* and *in vivo* evaluations is the only way to test its rational design.

Shown in Figure 2 are representative dendrimer scaffolds that have been seen in scientific literature with potential to be used as imaging and/or therapeutic agents for prostate cancer. Obviously, such dendrimer scaffolds can be also used for other cancer types and diseases.²⁹ In order to stay focused on the potential theranostic applications of dendrimers for prostate cancer, this review excludes the synthesis of the dendrimer scaffolds, which has been thoroughly discussed in recent reviews.^{23, 30}

STRATEGIES TOWARDS CONTROLLED DRUG RELEASE

Dendrimers, on account of their unique supramolecular and interfacial features, and chemically modifiable peripheral functional groups, are ideally suited as nanoscaffolds for bioactive agents. These nanoconstructs have successfully been utilized for delivery of drugs, genes, diagnostic dyes, and biologically active metal ions^{23, 31-35} by two general methods. One entails the encapsulation of functional agents within a dendrimer scaffold, the other deals with covalently linkage of active agents to the functional groups located on the branching units. In case of the encapsulation, the agents physically interact with the nanoscaffolds by non-covalent secondary binding processes, such as hydrogen bonding, van der Waals interactions, and electrostatic attractions between oppositely charged species located within the agents and the nanocarrier. Dendrimers, owing to the presence of a large number of surface terminal groups within the same molecule, are widely used to provide multivalency in biology and targeted drug delivery systems.^{18, 36} Multivalency arises from the multi-presentation of a functional molecule on a single platform, by which the interactions between the functional molecule and its biological targets can be significantly enhanced when compared to the individual bonding of an equivalent number of monovalent ligands to the same biological targets.³⁷ This phenomenon is widely used by biological systems, particularly for attachment,³⁸ signal transduction,³⁹ and cellular recognition.⁴⁰ Such enhancement termed as “multivalent effect” is driven by the high entropic gain in the formation of a multivalent complex. Dendrimer nanoscaffolds, owing to the vicinal presence of active functional groups within an adjustable distance, can easily mimic “ligand clustering” for the desired multivalent effect.⁴¹⁻⁴⁴

Non-covalent encapsulation

Dendrimers are capable of non-covalently harboring drugs, dyes, diagnostic agents, and other biologically active species. The secondary interactions (e.g. hydrogen bonding, electrostatic interactions, dipole-dipole, and hydrophobic interactions) between the dendrimers and bioactive agents are responsible for stabilizing of these agents. The resulting supramolecular self-assembly offers several distinct advantages such as enhanced solubilization of non-polar drugs in aqueous media and minimization of non-specific interactions of the encapsulated drugs with plasma components.

In the early 1990s, the synthesis and characterization of ‘dendritic boxes’ within poly(propyleneimine) dendrimers surrounded by a dense shell was published and adopted by other researchers in the development of drug delivery applications of dendritic polymers.^{45, 46} While the initial approaches dealt with non water-soluble systems, the subsequent dendritic molecules have been made water-soluble for the delivery of a wide variety of biologically active species with substantial therapeutic and pharmaceutical improvement. For instance, a full-and-half generation (G6.5) of PAMAM dendrimer was reported to aquate cisplatin, an effective anticancer drug. The non-covalently complexed cisplatin was proven more efficacious in the treatment of cancer.⁴⁷

The release of encapsulated drugs from their dendrimer scaffolds has been extensively studied as the responses to environmental conditions (e.g. pH, salts).⁴⁸⁻⁵⁰ For instance, a multifunctional dendrimer scaffold, which consists of diaminobutane poly(propylene imine) dendrimers (DAB) with poly(ethylene glycol) chains and guanidinium moieties on its exterior, was able to release the encapsulated pyrene upon the pH and/or osmotic environment change.⁴⁹

Covalent delivery with dendritic molecules

Covalently linking drug molecules to dendrimers is the commonly used approach in the construction of dendrimer drug delivery systems. Peripheral end-groups of a dendrimer can be used as attachment points to couple drugs to the core scaffold. The key to the design of a dendrimer-drug conjugate lies in the fact that multiple copies of the same or different drug molecules can be attached to each dendrimer molecule by various bioconjugation reactions between the orthogonal and complementary functional groups. Release kinetics of the active agents from the dendrimer scaffold is largely governed by the chemical linkage by which the drug is coupled. The major advantage of this approach is that chemistry strategies can be applied with hope to realize the goal of controlled release by taking advantage of the susceptibility of various chemical bonds to different *in vivo* conditions, such as the acidic tumor microenvironment, site-specific enzyme cleavage, or disease-specific metabolism alternation. Drugs have been linked to the dendrimer scaffold through amide,⁵¹ ester,⁵² hydrazone,⁵³ imine,⁵⁴ carbamate,⁵⁵ disulfide,⁵⁶ carbazate bonds,⁵⁷ and enzymatic cleavable peptide sequences.⁵⁸ Each of these linkages has their own differential mechanism of cleavage to separate the drug molecule from the scaffold. While ester, hydrazone, and carbamates depend on the surrounding pH for release, amides and peptidic sequences require an enzymatic involvement for their degradation and subsequent release of the active species.

Amide vs. ester bonds

Amide and ester bonds are most frequently used in the conjugation design of dendrimer-based drug delivery systems. In most cases, dendrimer-drug conjugates undergo the lysosomal or endosomal pathway after entering the cell.^{51, 59} In the acidic environment, the drug can be released from the dendrimer carrier upon the acidic cleavage of the ester bonds. However, amide bonds are robust and undergo a very slow enzymatic degradation. Owing to

their remarkable stability, the amide bond linkage is not the preferred for drug release.⁵¹ This drug release rate can also be affected by the different chemical environment of the same bond. For example, diethylene glycol (deg) and lactic (lact) ester linkages were assessed for their drug release rate from a G0 PAMAM dendrimer using naproxen (Figure 4), a prescription nonsteroidal anti-inflammatory drug with poor water solubility, as a model drug.⁶⁰ It was found that the deg linkage of G0-deg-NAP was much more quickly hydrolyzed than the lact ester bonds of G0-lact-NAP.

In order to understand the effect of architecture and linker on drug release, a recent study compared the drug release characteristics of a series of dendrimer-ibuprofen conjugates (see Figure 4 for the chemical structure of ibuprofen) built upon a G4 PAMAM dendrimer with ester, amide, and peptide as the drug and dendrimer linkage.⁵² As expected, amide-linked conjugates were relatively stable against hydrolysis, whereas the ester-linked conjugates showed a pH-dependent release rate. Interestingly, the conjugates constructed by direct amide and ester bonding did not release ibuprofen enzymatically in either cathepsin B buffer or diluted human plasma. In contrast, the dendrimer-ibuprofen conjugate incorporated with PEG as a linker released its drug loads rather efficiently by cathepsin B activity, so was the peptide-linked conjugates. This demonstrates that the steric crowding at the surface of dendrimer-drug conjugates along with the linking chemistries govern the drug release mechanisms as well as kinetics. Therefore, understanding these structural and steric effects on their drug release characteristics is crucial for the design of dendrimer conjugates. Shown in Figure 4, methotrexate (MTX), an anti-metabolite and anti-folate drug, has two possible sites for conjugation owing to the presence of carboxylic and amine terminations. As such, MTX can serve as a good drug model to compare the drug release profiles from amide and ester linkages. In fact, the carboxylate group of MTX is preferred in the construction of dendrimer-MTX conjugates because MTX can be released once the internalized conjugates are exposed to the acidic endosomal environment.^{61, 62}

Cis-aconityl bond

Cis-aconityl is another pH-sensitive linker that has been extensively exploited for the design and construction of drug delivery systems.^{63, 64} For instance, dendrimer conjugates of doxorubicin (DOX) constructed by use of cis-aconityl linkage was able to show an acid triggered DOX release, whereas the release from its amide counterpart was negligible.⁶⁵

Disulfide bond

The disulfide bond (-S-S-) is formed through the oxidation of two thiols. While relatively stable in mildly oxidizing environments (e.g. atmospheric oxygen or the blood stream), disulfide bonds can be cleaved by a reducing agent reforming two thiols.^{66, 67} In the cell, the reduced form glutathione (GSH) was maintained at the millimolar concentration level through the cytosolic NADPH-dependent reaction catalyzed by glutathione reductase. GSH is found at much higher level in diseased cells. For example, It was found to be 7-fold higher in a human lung adenocarcinoma cell line (A549) than in a normal human lung fibroblast line (CCL-210).⁶⁸ With this feature, the strategy of using disulfide bond linkage through reacting with sulfhydryl groups on dendrimer has been used to facilitate the drug release. Study showed by using this strategy the anti-inflammatory and antioxidant agent, *N*-acetyl-L-cysteine (NAC), can be tailored on dendrimer and released by the intracellular glutathione *in vivo*.^{69, 70} For PAMAM dendrimers possessing carboxylic or amine terminal groups, they must be first modified using glutathione or *N*-succinimidyl-(2-pyridyldithio)-propionate, respectively, and then coupled with the thiol moiety of NAC to form the dendrimer-drug conjugate through disulfide linkage. The construct was found to be able to avoid plasma protein binding, which is an additional advantage for the enhancement of drug bioavailability. Efficacy studies using this construct on microglial cells revealed that the

dendrimer-NAC conjugate was 16 times more efficacious than the drug alone in the treatment of maternal fetal infections.⁶⁹⁻⁷¹ Similar study was also done using thiol terminated star polyethylene glycol conjugates.⁷² Similar design was also seen in a paclitaxel (PTX) conjugate constructed from a G2 triazine dendrimer for the intracellularly controlled release of PTX for prostate cancer treatment.⁷³ The conjugate showed a better therapeutic efficacy as compared to its counterparts without the intracellular glutathione sensitive disulfide linker.⁷⁴

The conjugation methods mentioned above utilize diversified multifunctional and multivalent approaches and exhibited substantially positive biological results. Despite the success demonstrated by the conjugation methods, it must be noted that conjugating different classes of ligand molecules to the surface of the dendrimer scaffolds often results in a heterogenic distribution of ligand-bound dendrimer conjugates in terms of the number of ligands per conjugate. The ligand distribution on a dendrimer scaffold is related to the synthetic history of the dendrimer and the product conjugate becomes more inhomogeneous with increasing synthetic steps, as more ligands and ligand types are involved.⁷⁵ Such heterogeneous ligand distribution certainly would hinder the interpretation of biological and clinical results as the structure–activity relationship is compromised. To address this problem, efforts have been made through the implementation of new synthetic strategies and scalable purification techniques regarding the understanding of inhomogeneous ligand distribution.⁷⁶⁻⁸³

POTENTIAL CYTOTOXICITY OF DENDRIMER NANOSCAFFOLDS

Towards the eventual application as theranostic agents, the dendrimer nanoscaffolds must be able to maintain a certain degree of integrity under the physiological conditions, which provides desired cytotoxicity shielding of the carried therapeutic agents from non-target organs or tissues. As such, ideal nanoscaffolds themselves should not induce immune-responses, cause hemolytic toxicity, or impose cytotoxicity. However, non-modified or intact dendrimer scaffolds might elicit immune-responses or be toxic due to the size and the surface termination of the dendrimers. The otherwise exposed building blocks, if toxic, must be shielded by the surface termination or modification; and the dendrimer nanoscaffolds must remain stable because fragmentation would release the toxic building blocks, which may redistribute back to the blood stream and thus impose unnecessary harm to healthy tissues.

As exemplified by the surface-unmodified PAMAM dendrimer systems, the positively charged surface (quaternary ammonium ions) interacts with red blood cells and other healthy cells, which results in the hemolytic toxicity and cytotoxicity.⁸⁴ It has been reported that both hemolytic toxicity and cytotoxicity of PAMAM dendrimers are dependent on the charge, concentration, and generation of the dendrimers;^{85, 86} and lower generation PAMAM dendrimers (i.e. G0-G1) exhibit considerably less cytotoxicity than the higher generation ones⁸⁷ (i.e. G2-G4). On the other hand, both anionic and neutral PAMAM dendrimers are found substantially less toxic than cationic dendrimers.⁸⁸ Based on these observations, surface chemistries play an essential role in the design of a dendrimer nanoscaffold system in order to reach the desired biocompatibility. Usually two common approaches are taken: 1) surface modification by polyethylene glycol (PEGylation) to neutralize the surface charge and improve the water solubility of the dendrimer system; and 2) surface termination with small molecules (e.g. acetylation) to neutralize the surface charge and enhance the cellular uptake.⁸⁹ It has been well recognized that surface PEGylation renders nanoconstructs with desired stealthiness to the immune system and biocompatibility. In addition, long PEG chains are usually employed to optimize the *in vivo* kinetics and increase the biological half-lives of nanoconstructs.^{90, 91} A good example of the

latter was set by a study on the effect of surface amidation of PAMAM dendrimers on their cytotoxicity,⁸⁸ in which a linear relationship was found between the number of the naked surface amine groups and the cytotoxicity of dendrimers while the desired transepithelial permeability was not compromised. Not surprisingly, the complete amidation led to a non-toxic dendrimer system.

Fatty acids, such as lauric acid (namely, dodecanoic acid: $n\text{-C}_{11}\text{H}_{23}\text{COOH}$), can also be used to modify dendrimer surface as a biocompatible penetration enhancer.⁹² It was reported that lauroylation of cationic PAMAM dendrimers reduces the cytotoxicity along with the surface PEGylation and the degree of lauroylation can be used as an approach to improve the dendrimer's cell permeability.⁹³ Of course, further modification of a dendrimer nanoscaffold system with targeting or other functional molecules must take into consideration its effect on the biocompatibility of the system.

CONSTRUCTION OF TARGETED DENDRIMER NANOSCAFFOLDS

Targeting is important in that it can provide higher contrast to imaging of the targeted organ or more efficacious therapy to the specific diseased sites. Tumor targeting of nanoconstructs can be achieved by either passive or active targeting mechanisms. The passive-targeting approach takes advantage of the enhanced permeability and retention (EPR) effect,⁹⁴⁻⁹⁶ which resulted from the rapid tumor growth during tumor angiogenesis because a defective vascular architecture is required to sustain the demand for adequate nutrient and oxygen supply as well as the waste disposal.⁹⁷ The defective vasculatures are characterized by unusually large gaps (ca. 300-800 nm) between adjacent endothelial cells of the tumor blood vessels resulted from an abnormal arrangement of basement membrane and perivascular cells.⁹⁸ This led to the enhanced permeability of the blood vessels of solid tumors to blood-borne macromolecules or nanoparticles. However, it should be also remembered that the normally high interstitial pressure in angiogenic tumors along with the chaotic and disorganized neovasculatures may partially offset the extravasation of particles caused by the EPR.⁹⁷ Nonetheless, lack of an effective lymphatic drainage system,⁹⁹ solid tumors can accumulate nanoconstructs that leak out of the permeable blood vessels, especially the ones stealthy of RES sequestration.^{100, 101} Many drug-loaded dendrimer systems have been reported with efficient uptake in prostate cancer for therapeutic treatment by taking advantage of this passive targeting mechanism.^{73, 74}

Active targeting strategies normally exploit the existence of specific molecular signatures of targeted sites. In general, tumor cells of a specific cancer type over-express specific receptors or antigens. It would be straightforward to conjugate nanoconstructs with the specific ligands of the receptors or antigens for active targeting, which may allow early diagnosis, patient stratification, or personalized therapy at molecular level while alleviating cytotoxicity to normal tissues.¹⁰² To date, varieties of nanoconstructs have been functionalized with small molecules, peptides, and antibodies to target endothelial receptors on the microvasculature of proliferating tumors.¹⁰³⁻¹⁰⁷ Here we focus our discussion on two commonly exploited targets for targeted imaging or therapy of prostate cancers: Prostate-Specific Membrane Antigen (PSMA) and $\alpha_v\beta_3$ -integrin.

Targeting the Prostate-Specific Membrane Antigen (PSMA)

Prostate specific membrane antigen (PSMA) is a type II transmembrane glycoprotein that is over-expressed in prostate cancer and neovasculature, but not in the vasculature of the normal tissues.^{108, 109} A cell surface protein that presents a large extracellular domain (amino acids 44-750), PSMA has been utilized as an effective target for monoclonal antibody directed imaging agents or therapeutics for prostate cancer.¹¹⁰⁻¹¹³ Indeed, the only FDA approved prostate cancer imaging agent is an ¹¹¹In-labeled PSMA monoclonal

antibody (7E11-C5.3).^{110, 112} Clinical trials showed that SPECT imaging with this agent had improved sensitivity in the detection of prostate cancer compared to CT or MRI, and it is in clinical use to define the stage of localized prostate cancer and metastases in conjunction with CT or MRI.¹¹² However, recent reports indicate problems in terms of imaging specificity and sensitivity given the fact that the antibody (7E11-C5.3) recognizes an internal epitope of PSMA.¹¹⁰ To address the problems, many monoclonal antibodies have been developed (e.g. J591/MLN591, J533, J415, E99, and E6 etc.) that recognize the extracellular domain of PSMA.^{111, 114-118} As anticipated, imaging with these antibodies labeled radioisotopes have shown significantly improved sensitivity and specificity in the detection of prostate cancer. These antibodies have also been seen in the construction of targeted dendrimer systems. For instance, a PAMAM dendrimer conjugate with J591 showed the specific binding to the PSMA⁺ LNCaP cells but not the PSMA⁻ PC-3 cells.¹¹⁹

Targeting PSMA can also be achieved by using aptamers, a class of nuclease-stabilized oligonucleotides selected by a ligand screening technology, SELEX (systematic evolution of ligands by exponential enrichment).¹²⁰⁻¹²⁷ Much smaller than antibodies and without immunogenicity, aptamers might be able to contest the roles of antibodies in therapeutics and diagnostics. In 2002, two RNA aptamers, xPSM-A9 and xPSM-A10, were identified with high binding affinity to the extracellular domain of PSMA. The first PSMA-targeted aptamer-nanoparticle conjugate was built from xPSM-A10. It was found that the nanoconjugate can efficiently target and accumulate in PSMA⁺ LNCaP cells but not in PSMA⁻ PC-3 cells.¹²⁸ Recently, a G4 PAMAM dendrimer was reported as a carrier of A10 aptamer-DOX complex for prostate cancer treatment;¹²⁹ and the PSMA-targeted dendrimer system displayed an antitumor efficacy comparable to DOX alone. Similarly, a G5 PAMAM dendrimer conjugate with A10-3.2, a truncated form of xPSM-A10, was reported with capability of delivering tumor suppressor genes to PSMA expressing target.¹³⁰

Targeting $\alpha_v\beta_3$ -integrin

The progression rate of a solid tumor is strongly dependent on its ability to stimulate angiogenesis, a process in which new blood vessels grow from pre-existing vessels to supply tumor cells with oxygen and essential nutrients. Integrin adhesion receptors are the key factor for cell to interact with the extracellular matrix (ECM)¹³¹ in order to activate the signaling pathways for regulating the cell functions such as motility, proliferation, and differentiation.⁴¹ In 1994, $\alpha_v\beta_3$ -integrin, the vitronectin receptor, was found highly upregulated on activated endothelial cells with a critical role in their survival, while its expression is weak in most normal organ systems.¹³² To date, $\alpha_v\beta_3$ -integrin has been well recognized as a receptor affecting tumor growth, local invasiveness, and metastatic potential.¹³³ Among over 25 identified integrin receptors, about two thirds including $\alpha_v\beta_3$ -integrin recognize and bind tightly to a tripeptide sequence Arg-Gly-Asp (RGD). In addition to numerous synthetic RGD peptido-mimetics, RGD-containing peptides, usually in the cyclized form, have been developed as $\alpha_v\beta_3$ antagonists with potential to decrease angiogenesis and induce tumor regression and apoptosis within the angiogenic blood vessels.^{132, 134-140} To date, cyclic RGD peptides and peptidomimetics have been extensively explored for $\alpha_v\beta_3$ -integrin targeted imaging and therapy. The first $\alpha_v\beta_3$ -integrin targeted nanoconstruct was built upon a polymerized lipid-based vesicle conjugated with an $\alpha_v\beta_3$ antagonist for targeted gene delivery to angiogenic blood vessels. Tumor cell apoptosis and regression was observed after the nanoconjugate administration to treat established primary and metastatic cancers.¹⁰⁵

In prostate cancer, the expression of $\alpha_v\beta_3$ -integrin is commonly observed in several cell lines including DU145 and PC-3. Interestingly, it was found that the adhesion and migration of cancer cells was mediated partly by $\alpha_v\beta_3$ -integrin during distal metastasis of prostate

cancer. Not surprisingly, $\alpha_v\beta_3$ -integrin targeted dendrimers have been reported with potential for targeted imaging and therapy of prostate cancer.^{141, 142}

IMAGING APPLICATION OF DENDRIMER NANOSCAFFOLDS

Given the unique structural features, dendrimers have been extensively utilized for molecular imaging research. The imaging application of dendrimer nanoscaffolds is mainly based on the facts: 1) the versatility of surface chemistries can tailor a common dendrimer scaffold for different applications and imaging signal amplification if necessary; 2) functionalities can also be incorporated into the core and the interior radial structures; and 3) most dendrimer scaffolds are robust under physiological conditions. In this section, we briefly discuss the imaging applications of dendrimer scaffolds categorized by common imaging modalities.

Magnetic Resonance Imaging (MRI)

With exquisite spatial resolution and superior soft tissue contrast, MRI holds great promise in the diagnosis of prostate cancer. However, MRI with gadolinium-based contrast agents is inherently less sensitive as compared to PET or SPECT. This is where dendrimer scaffolds can come to play an important role. Earlier efforts were made on linear and hyperbranched polymers, to which multiple copies of contrast agent were attached for the desired signal enhancement.^{143, 144} However, their *in vivo* applications were hampered by the inherent polydispersity and inefficient renal excretion of the polymer conjugates. In contrast, dendrimer scaffolds are monodisperse and their sizes are tunable for optimal biodistribution.²³ In addition, dendrimers can also be retained in solid tumors through EPR effect, where the Gd(III)-based T_1 relaxivity can be further enhanced via the confined moving and tumbling. To date, dendrimers have been reported for both T_1 and T_2 contrast agents: 1) dendrimers containing superparamagnetic iron oxide particles (SPIO) (magneto-dendrimers) and 2) dendrimer incorporated with high numbers of Gd(III)-chelates.^{145, 146} The former is exemplified by a G4.5 PAMAM dendrimer system loaded with iron oxide particles¹⁴⁷ with potential to be used as a cell tracking agent for monitoring the fate of stem cells. Such magneto-dendrimer scaffolds have also shown utility in gene delivery into cells. Interestingly, the cell uptake level of DNA-loaded cationic magneto-dendrimers can be dramatically enhanced in the presence of magnetic field due to the magnetic nature.¹⁴⁸ For instance, a G6 PAMAM dendrimer loaded with SPIO was used to transfect COS 7 cells.¹⁴⁹

Compared to the magneto-dendrimers, Gd(III)-complex loaded dendrimers were much more widely used. Conjugation of Gd(III) complexes to dendrimer's peripheral functional groups results in significant enhancement of relaxivity. For example, the r_1 relaxivities of G4 and G5 PAMAM dendrimers conjugated with Gd-DTPA (DTPA: diethylenetriaminepentaacetic acid) can be considerably enhanced (G4: $r_1 = 28 \text{ mM}^{-1}\text{s}^{-1}$; G5: $r_1 = 30 \text{ mM}^{-1}\text{s}^{-1}$) as compared to the low molecular weight Gd-DTPA (Magnevist®: $r_1 = 5.5 \text{ mM}^{-1}\text{s}^{-1}$). It is noteworthy that the proton relaxivity of the Gd(III)-loaded dendrimers are not enhanced proportionally as the dendrimer generation increases (G8: $r_1 = 35 \text{ mM}^{-1}\text{s}^{-1}$).²³ This can be attributed to the decreased water accessibility of Gd(III) in the high-generation dendrimer structure. Therefore, the G6 PAMAM dendrimer scaffold is the most frequently used for the r_1 enhancement of Gd(III)-based contrast agents.¹⁵⁰⁻¹⁵³ Inherent from the nature of dendrimers, different generations of dendrimer can be used for different applications. As such, different generations of Gd(III)-dendrimer contrast agents have been reported for different imaging applications: drainage lymph node, vasculature (angiograph), kidney, tumor, or even whole blood pool.²³

Computed Tomography (CT)

An inherent anatomical imaging technique, CT provides excellent hard tissue contrast. To enable x-ray based angiography, a contrast agent that efficiently absorbs x-rays is needed through a systemic administration. In sharp contrast to the huge volume of reports using dendrimer scaffolds for MRI signal enhancement, few such efforts have been seen for CT contrast agents. The typical synthesis of CT contrast agents constructed from dendrimer scaffolds was seen in a recent report, in which a series of G3, G4, and G5 dendrimers with a PEG core were conjugated with tri-iodophthalamide moieties to the peripheral amino groups.¹⁵⁴ Among them, the G4 dendrimer conjugate showed the optimal intravascular contrast in a rat model, while all conjugates exhibited adequate water-solubility, low osmolality, and good chemical stability.

Optical imaging

Optical imaging's popularity in molecular imaging can be partially attributed to the common use of a charge-coupled device (CCD) camera, which is portable and relatively inexpensive. In addition to its inherent high sensitivity,^{155, 156} it has excellent temporal and spatial resolution.¹⁵⁷⁻¹⁵⁹ Even though the limited depth of tissue penetration of the light signals makes optical imaging cannot compete with PET, MRI, and CT in clinical applications, it still holds great potential for understanding the disease progression and therapeutic evaluation at preclinical level. Detail for the applications of optical imaging can be found in many review articles.¹⁶⁰⁻¹⁶⁵ Because of the inherent high sensitivity of optical imaging, it is often unnecessary to apply a nanoconstruct to enhance its signal readout. In most cases, optical imaging probes were incorporated into a nanoscaffold for a secondary purpose, such as *ex vivo* validation of *in vivo* imaging results obtained from other imaging techniques.^{150, 166, 167} For instance, a dual imaging probe can be constructed from a G5 dendrimer scaffold by conjugating three imaging reporters to the surface amine groups – two fluorophores (Cy5.5 and Rhodamine) for optical imaging and fluorescence microscopy, Gd(III)-DOTA for MRI, and a targeting molecule (angiopep-2) for transcytosis and glioblastoma targeting. The resulting nanoprobe was able to cross the blood brain barrier and reach the target for optical and MR imaging of glioblastoma.¹⁶⁸

Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT)

PET and SPECT are two main nuclear imaging techniques that are capable of providing tomographic and quantitative functional information inside a living subject.^{169, 170} In theory, natural nonradioactive substance in an organism's physiological and biochemical processes can be measured by its radioactive counterpart based on the tracer principle. In reality, most PET and SPECT procedures are performed with radiolabeled compounds, called radiopharmaceuticals or radiotracers or nuclear imaging probes, which rarely have their biological counterparts. The physical difference of PET and SPECT in the use of radioisotopes dictates the types of hardware and software for the detection, localization, and quantification of the decay events. While PET detects pairs of 511 keV γ -rays resulted from the annihilation of positions emitted from the PET imaging probe with electrons, SPECT acquires γ -rays directly emitted for the radiotracer. Unlike visible light, γ -rays can easily pass through the body and be detected for quantitative imaging construction and data analysis. The commonly used radioisotopes in PET and SPECT are listed in Table 1.

Dendrimer nanoscaffolds have been commonly seen in PET and SPECT studies.^{26, 194} The purpose of incorporating a nuclear imaging reporter into the nanoconstructs is to track the *in vivo* distribution and quantify the targeted delivery of the nanoconstruct system. The conjugation chemistry approach is virtually identical to what have been described above. The only difference is that different radioisotopes of choice require their corresponding

chelators or labeling functionalities (Table 1). This is based on the concept that the radiolabel moiety must remain stably attached to the nanoconstructs, otherwise the imaging readouts will not reflect the biological behavior of the nanoconstructs. It is noteworthy that dendrimer scaffolds conjugated with 1,4,7,10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) can be labeled with many different radioisotopes, which include ^{177}Lu , ^{111}In , ^{67}Ga for SPECT imaging^{179, 183, 194} or ^{68}Ga , ^{64}Cu , and ^{86}Y for PET imaging.¹⁹⁵⁻¹⁹⁷ When considering a proper radioisotope for a specific application, the biological half-life of the nanoconstruct determines the radioisotope options. For instance, a low-generation dendrimer (G0-G3) that has a rapid clearance profile through kidneys should be labeled with a short-lived radioisotope, such as ^{68}Ga or ^{64}Cu . In contrast, a high-generation dendrimer (> G7) with 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.

Among the noninvasive imaging techniques, PET or SPECT is exceptionally sensitive but with poor spatial resolution, while MRI has high spatial resolution and exquisite soft tissue contrast but with low sensitivity. Therefore, the synergistic combination of PET/MRI or SPECT/MRI will certainly be advantageous over MRI, PET or SPECT alone.¹⁹⁸ To date, integrated PET/MRI scanners have been successfully implemented with potential to become the mainstream of molecular imaging in near future. For PET/MRI or SPECT/MRI, imaging agents that enable both imaging procedures could provide complementary information for a better diagnosis. However, the major challenge is how to overcome the large difference in sensitivity of the two techniques. Nanoparticle-based agents have been extensively pursued in this regard. Obviously, the design and synthesis of such dual-modality imaging probes can be readily realized by attaching bifunctional chelators to the surface of dendrimers loaded with superparamagnetic iron oxide (SPIO) or Gd(III) chelates. Information obtained from the dual-modality imaging of PET/MRI are anticipated to enable the perfect co-location and cross-validation of MRI and PET agents in the target regions of interest. While the MRI scan provides the anatomical information, motion artifact correction, and PET partial volume correction, the PET scan can be used for better imaging quantification so that higher detection sensitivity can be achieved with more accurate molecular signature changes over the course of study.¹⁹⁹⁻²⁰¹

THERAPEUTIC APPLICATIONS OF DENDRIMER NANOSCAFFOLDS

Dendrimer nanoscaffolds have been used to carry a variety of therapeutic agents for either passively or actively targeted delivery with the aim to improve the therapeutic efficacy of cancer treatment. The design and synthesis of therapeutic dendrimer-based drug delivery systems takes a similar methodology as described in the previous sections. The major design concerns include the biocompatibility of the nanoconstructs, bioavailability of the loaded therapeutics (controlled release), and the *in vivo* kinetics of the dendrimer-based drug delivery system. In this section, we briefly discuss the therapeutic applications of dendrimer scaffolds categorized by common therapeutic interventions.

Chemotherapy

To date, many chemotherapy drugs have been seen in the construction of dendrimer-based drug delivery vehicles, which include Curcumin,²⁰²⁻²⁰⁵ Genistein,²⁰⁶ *cis*-diamminedichloroplatinum (Cisplatin),^{147,188} PTX,^{73, 74} resveratrol,²⁰⁷ DOX,¹²⁹ camptothecin (CPT),^{148, 149} and MTX.^{40, 150} Curcumin has shown potential to suppress *in vitro* prostate cancer cell proliferation in both androgen-sensitive prostate cancer cell line LNCaP and androgen-independent cell line DU145;²⁰²⁻²⁰⁵ and *in vivo* tumor growth in a LNCaP xenograft mouse model.²⁰³ Similar inhibitive effects have been seen for Genistein to suppress prostate cancer growth.²⁰⁶ The purpose of constructing a dendrimer-based drug

delivery system is to improve the bioavailability and increase the payload of the drugs so as to achieve a more efficacious therapy while reducing the toxic effects on healthy tissues. It was found that the interior size of a dendrimer system plays an important role in the non-covalent packing of drugs. For instance, a high-generation dendrimer (G7-G9 PAMAM) has a tight structure, which provides less accessibility of drug molecules to the interior space. As such, a lower generation of PAMAM dendrimers (G4 – G6) is preferred for the non-covalent loading of drugs into the interior space of the dendrimer (Figure 1).²⁹ Structural analysis showed that Cisplatin was able to bind to PAMAM dendrimers through the interaction between Pt cations and the hydrophilic region of peripheral amino groups.²⁰⁸ PAMAM dendrimers were reported with great potential to encapsulate many chemotherapeutic drugs such as Curcumin, Genistein and Resveratrol via non-covalent interactions. It was found that the encapsulation efficiency as measured by the binding affinity increases from G4-PAMAM to G4-PAMAM with PEG to G3-PAMAM with PEG, while the drug release rate was in the order of Curcumin > Cisplatin > Genistein > Resveratrol.²⁰⁸ Hence, PAMAM-based dendrimer scaffolds are able to carry both hydrophilic and hydrophobic drugs depending on the spatial geometry of the dendrimer scaffolds. Poly(glycerol succinic acid) dendrimers (PGLSA dendrimers) were investigated to encapsulate hydrophobic CPTs, in which the peripheral groups of G4-PGLSA dendrimers were found with an important role in the encapsulation of a camptothecin analog, 10-hydroxycamptothecin (10-HCPT).²⁰⁹ While the G4-PGLSA dendrimer bearing hydroxyl groups (G4-PGLSA-OH) failed to encapsulate the drug molecules, the one with carboxylate groups (G4-PGLSA-COONa) succeeded. The G4-PGLSA-COONa dendrimer could also be loaded with 7-butyl-10-aminocamptothecin (BACPT). Encouragingly, both drug-encapsulated G4-PGLSA-COONa dendrimers showed a significant improved anticancer activity to various cancer cell lines.²¹⁰ The current challenge is how to overcome the lack of controlled release mechanisms.

In addition to the encapsulation approach, covalent linkages can also be utilized for drug loading. As discussed in the chemistry strategies of controlled drug release, chemical bonds selectively labile to different *in vivo* conditions are required. Given the two available conjugation sites of MTX, it can be non-valently linked to a dendrimer scaffold through either carboxylate or amine groups.²¹¹ While both dendrimer-MTX conjugates were formed by the amide linkage, the former showed an appreciable sensitivity increase against an MTX-resistant human leukemic lymphoblasts cell line, while no sensitivity increase was observed for the latter. This difference of therapeutic efficacy might be attributed to the dendrimer's surface charge after the release of MTX. Other dendrimer-drug conjugate systems were also reported for cisplatin,²¹² indomethacin,²¹³ and paclitaxel.^{74, 214}

Gene therapy: suicide gene, siRNA, and microRNA

Gene therapy is a therapeutic approach with the potential for prostate cancer therapy. However, with over 90 clinical protocols of gene therapy, only four of them progressed to phase III.²¹⁵ By far, viral systems are the most effective gene delivery carriers, but the safety concerns (e.g. acute toxicity, immunogenicity, and oncogenicity, etc.) remain the biggest hurdle for the translational and clinical trials.^{216, 217}

Recently, a non-viral delivery system constructed from a G5 PAMAM dendrimer was reported for herpes simplex virus (HSV)-thymidine kinase (TK)/ganciclovir (GCV) and connexin43 (Cx43) duo-suicide gene therapy. The G5-PAMAM conjugate delivered suicide genes TK-Cx43 led by a PSMA promoter; and this double-targeted and double-enhanced system was demonstrated being effective in inducing cell growth inhibition and apoptosis *in vitro* and suppressing tumor growth *in vivo* in PSMA⁺ LNCaP tumor but not in PSMA⁻ PC-3 tumor.²¹⁸

RNA interference (RNAi) has emerged as a new promising approach for gene therapy. RNAi is a sequence-specific gene silencing process which is controlled by the RNA-induced silencing complex (RISC). With the specific sequence against the complementary mRNA, small interfering RNA (siRNA) can trigger RNAi activity, interfere mRNA translation, and further block the downstream protein synthesis. Given the great potential of this approach for gene therapy, tremendous efforts have been seen to apply available methods for siRNA delivery.²¹⁹⁻²²² Of them, a cell-internalizing aptamer has shown great promise for gene therapy of prostate cancer by directing the siRNA delivery system to PSMA.²²³ Recently, a PAMAM dendrimer with a triethanolamine (TEA) core was shown with the ability to directly serve as the vector of a siRNA targeting heat shock protein 27 (Hsp27),²²⁴ an ATP-dependent molecular chaperone up-regulated during the hormone ablation and chemotherapies of prostate cancer treatment. The inhibition of Hsp27 expression leads to the increased level of caspase 3-dependent apoptosis thus suppressing tumor growth rate.^{225, 226} Through the self-assembly of the siRNA by adding complementary RNA with A_n/T_n 3'-overhangs, a lower-generation PAMAM dendrimer was found with potential to assemble siRNA for intracellular delivery. With this effective siRNA delivery system, silencing of Hsp27 gene was observed along with activated caspase 3-dependent apoptosis and inhibited tumor cell proliferation in a PC-3 xenograft mouse model (Figure 5).²²¹

Although siRNA has been widely used, it is difficult to suppress the cancer progression via blocking a single gene activity. Recently, the discovery of microRNAs (miRNAs) has advanced RNAi into the mechanistic research of gene regulation.²²⁷ A group of endogenous non-coding RNAs, microRNAs are able to either activate or inhibit protein translations.²²⁸ Recent evidence indicates that miR-15a and miR-16-1 act as tumor suppressor genes in prostate cancer by down-regulating the expression of survival genes such as bcl2, ccnd1, and wnt3A.²²⁹ When functionalized with maleimide groups, a G5 PAMAM dendrimer was reported with capability to deliver thiolated miR-15a and miR-16-1 into prostate cancer cells to induce the cell death.¹³⁰ This provides a new promising method to develop novel therapies against advanced prostate cancer.

Immunotherapy and Radioimmunotherapy

Due to the intrinsic low proliferation of prostate cancer, chemotherapy drugs aimed to ablate cancer cells with high proliferation index might not be effective. With the success of immunotherapy using Rituximab (anti-CD20) and Trastuzumab (anti-erbB2), monoclonal antibodies have become one of the fast-growing classes of cancer therapeutics,²³⁰ which involves several prostate cancer related targets (e.g. prostate-specific antigen, mucin, and PSMA), and other cell surface receptors such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2/neu).²³¹ Cancer vaccine has also shown potential in prostate cancer therapy. To date, several prostate cancer associated antigens have been identified, which can serve as targets for vaccine development.²³² Moreover, by combining cancer vaccination and hormonal ablation therapy, a considerable expansion of vaccine-induced effector cells was observed. Therefore, active immunotherapy against prostate cancer might be more potent than the traditional chemotherapy against prostate cancer after androgen ablation treatment.²³³ Not surprisingly, dendrimer has been used for vaccine delivery.²³⁴

Radioimmunotherapy is a straightforward extension of immunotherapy when the monoclonal antibody is labeled with a therapeutic radioisotope, such as ¹⁷⁷Lu, ⁹⁰Y or ¹³¹I.²³⁵⁻²³⁷ The chemistry strategies involved in radioimmunotherapy are nearly identical to the approaches discussed in the imaging applications.

Cell receptor-based therapies also include the use of peptides or other small targeting molecules, which can be readily and cost-efficiently identified by combinatorial library

approaches and synthesized by solid phase chemistry modules. Unlike monoclonal antibodies, these small receptor-specific ligands do not elicit immune-responses and can withstand harsh conditions for chemical modifications. Because small molecules often exhibit rapid *in vivo* kinetics, conjugating them with a dendrimer nanoscaffold is a convenient way of developing them for targeted cancer therapeutics.

TOWARDS DENDRIMER-BASED THERANOSTICS OF PROSTATE CANCER THROUGH RADIOISOTOPE INCORPORATION

From the perspective of nuclear medicine, theranostics is a just new compound word. The concept and practice in nuclear medicine can be traced back to 1940s because of the straightforward dual use of radioisotopes that emit both β^- particles for therapy and γ -rays for scintigraphic diagnosis as in the routine clinical practice of using radioiodine nuclides for differentiated thyroid therapy. Indeed radioisotope imaging allows noninvasive and longitudinal assessment of drug delivery and thereafter treatment efficacy, which can also be elicited by radiotherapeutic particles emitted by the radioisotope itself. Similar radioisotopes to radioiodine are commonly seen in conventional nuclear medicine. For instance, both ^{186}Re ($t_{1/2} = 3.78$ d) and ^{188}Re ($t_{1/2} = 16.9$ h) can be used for SPECT imaging (γ -energies: 137 keV and 155 keV, respectively) and radiotherapy (β^- emissions: 1.07 MeV and 2.12 MeV, respectively) (Table 1).¹⁸⁸⁻¹⁹³ Conveniently available from a commercially available generator, ^{188}Re is more commonly seen in research and clinical practice. The pair of ^{186}Re and ^{188}Re can be judiciously chosen for the radiotherapeutic treatment of solid tumors with different sizes. While ^{188}Re is optimal for the radiotherapy of large tumors, the lower β^- emission energy makes ^{186}Re more suitable for small tumor treatment. To date, both ^{186}Re and ^{188}Re have been found in the construction of radioactive nanoconjugates.^{192, 238-240} For instance, ^{186}Re -labeled liposomes loaded with doxorubicin was reported for SPECT imaging and chemoradionuclide therapy of cancer in a head and neck squamous cell carcinoma xenograft rat model.¹⁸⁸ Multiple SPECT images acquired in a 120-h period showed the *in vivo* kinetics of the radioactive nanoconjugate, which had slow blood clearance, low liver uptake, an increasing deposition in both spleen and tumor. When labeled with ^{188}Re , PEGylated liposome-based nanoconjugates of doxorubicin were reported with therapeutic potential in various cancer animal models,²⁴¹⁻²⁴⁶ indicating that nanoscaffolding is a practical means to afford desired pharmacological and targeting properties for imaging and therapeutic agents.

Lutetium-177 ($t_{1/2} = 6.71$ d) is also a radioisotope emitting both γ -rays (208 keV; 11%) and β^- particles (0.5 MeV), which can find applications in the development of theranostic agents. Obviously, approaches as described above are applicable to ^{177}Lu and they have been seen in the literature. Mostly recently, a ^{177}Lu -labeled nanoconjugate constructed from C_{80} fullerenes (^{177}Lu -DOTA-f- $\text{Gd}_3\text{N@C}_{80}$) was reported as a theranostic agent to deliver effective interstitial brachytherapy in two orthotopic xenograft brain tumor models of glioblastoma multiforme (GBM).²⁴⁷ Although the SPECT imaging potential with ^{177}Lu -DOTA-f- $\text{Gd}_3\text{N@C}_{80}$ was not performed or mentioned in the paper, it can definitely provide a noninvasive and quantitative tool to monitor the delivery and therapeutic efficacy of the radioactive nanoconstruct.

Obviously, other radioisotopes including ^{64}Cu and ^{111}In that emit positrons or γ -rays for PET or SPECT imaging and β^- or Auger electrons for radiotherapy can be simply used to construct theranostic agents in the form of either small molecules or nanoconjugates.

However, although with straightforward approaches for the design and synthesis of radioactive nanoconstructs for theranostic agent development, using radioisotopes is not always considered as an ideal way in this endeavor. Unnecessary radiation exposure and

public fear of radiation are among the main concerns. Another major issue with the radioisotope-enabled theranostics is that radioactive decay is always going on and irradiates the whole body when systemically administered. From the perspective of *in vivo* pharmacokinetics, radioisotope-labeled nanoconjugates might not be able to find a practical application in clinics because of the excessive irradiation to non-target organs resulted from the inevitable passive accumulation in the RES organs and the long blood circulation half-life of the nanoconjugates unless they can be efficiently cleared from kidneys.^{248, 249} On the other hand, small molecules with organ-specific targeting property and an efficient clearance profile can be well positioned in the development of radioisotope-enabled theranostic agents as in the conventional nuclear medicine practice with radioiodine.

The design and synthesis of theranostic agents other than radioisotope-enabled ones remains challenging. In this regard, nanoscaffolds are advantageous over small molecules in that a nanoscaffold typically presents multiple functionalities and a high potential payload for functional molecules. In other words, the capability of multi-presentation of a molecule and multiplexing of different functionalities makes nanoscaffolds an appealing class of carriers for targeted delivery of both diagnostics and therapeutics in a single entity, namely a theranostic agent. As discussed above, given the unique structural features and proven potential in both imaging and therapy applications, dendrimers are a practical class of nanoscaffolds that can find active roles in the development of theranostics. However, in spite of the functional versatility of dendrimers, a theranostic agent cannot simply be prepared by presenting three different functionalities (diagnostics, therapeutics, and targeting vector) on a single dendrimer nanoscaffold. Although a dendrimer system has a well defined structure, presenting different functionalities may incur unexpected chemical interference between different functional groups or regions, which would result in a failed or an irreproducible reaction. From the perspective of chemistry, an ideal dendrimer nanoscaffold should present different functional groups with a defined stoichiometry for orthogonal functionalization so as to achieve the designed theranostics reproducibly in a controlled manner. Such examples exist in the literature. For instance, a Janus-like dendrimer scaffold formed by combining two separately synthesized dendrons was recently reported. Shown in Figure 6, this Janus-like dendrimer system presents different functionalities with defined stoichiometry: 9 azide, 9 amine, and 54 carboxylate groups,⁷⁸ which enabled an orthogonal functionalization to present the defined number of each functional molecule for a potential theranostic agent.

The systematic development of theranostic agents based on nanoscaffolds is still in its infancy, although promising examples have been shown in the literatures. To illustrate the basic concept and current status of nanoscaffold-based theranostic agent development, we summarized the key components that have been used for the construction of theranostic agents in Table 2.

In summary, while advances in the field of theranostic agent development are obvious, challenges still remain to be addressed even in the preclinical development status: 1) Dosage difference between the components of therapeutics and diagnostics if a dual-use radioisotope is used; 2) Passive uptake in RES organs; 3) Toxicity and aggregation of dendrimer scaffolds after functionalization; 3) Bioavailability or controlled release of loaded therapeutics; 4) Different requirements of *in vivo* pharmacokinetics for therapeutics and diagnostics; and 5) Administration routes and dosing plan for optimal diagnostic value and therapeutic efficacy. The FDA regulations governing the diagnostic and therapeutic agents are expected to become the main hurdles when a nanoscaffold-based theranostic agent reaches the translational status toward the eventual applications in human trials. For small molecular theranostics enabled by dual-use radioisotopes, especially those that have been in FDA's approval, the translational human trials could be more straightforward.

Knowing the status of molecular target(s) of disease before, during, and after therapy, theranostic agents are expected to play a critical role in personalized medicine. While challenges remain, the perspective of theranostic agents is promising. The future realization of clinical theranostic practice requires tenacious multi-disciplinary efforts from chemists to oncologists and cross-specialty coordination between radiologists and oncologists in today's preclinical development.

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Figure 1. A dendrimer system often has tunable physical properties for different biomedical applications, which are mainly determined by the dendrimer’s generation. This unique feature depicted here is represented by a PAMAM–DTPA (Gd) system (G0–G9) that was developed for MRI contrast agents. Different dendrimer systems may possess different tunable physical properties. The figure was adapted from reference Menjoge, A.R. et al.²³

Figure 2.

Structures of the dendrimer systems that have been used for the development of diagnostics and therapeutics of prostate cancer. PAMAM: poly(amidoamine); Bis-MPA: 2,2-bis(hydroxymethyl) propionic acid; PPI: polypropylenimine; PEG: Polyethylene glycol; 5-ALA: 5-aminolaevulinic acid; TEA: triethanolamine.

Figure 3.
Two general methods of loading active drug to dendrimers: a) non-covalent encapsulation and b) covalent conjugation.

Figure 4.
Structures of selected drugs used for construction of dendrimer therapeutics.

Figure 5.

Self-assembly of siRNAs on a dendrimer scaffold for gene therapy of prostate cancer. The sticky siRNAs of heat shock protein 27 (Hsp27) bear complementary An/Tn ($n = 5$ or 7) 3'-overhangs (An : yellow; Tn : red). The overhangs serve two roles in this siRNA delivery vehicle design: facilitating the self-assembly of the siRNAs and non-covalently anchoring the siRNAs at the dendrimer scaffold for delivery. Reprinted with permission from *Molecular Pharmaceutics*.²²¹

Figure 6.

A Janus-like dendrimer system with potential to serve as scaffold to present stoichiometric multi-functionalities for the development of theranostic agents. The dendrimer core is depicted in dark red; azide groups in purple; amine termini in red; and the terminal acid groups in green. Reprinted with permission from *Organic Letters*.⁷⁸

Table 1

Radioisotopes of interest for the development of dendrimer-based diagnostics and therapeutics for prostate cancer.

Radioisotope	Half-life (t _{1/2})	Decay Mode	Labeling moieties	Principal Applications	Ref.
⁶⁴ Cu	12.7 h	β ⁺ (17.4%) γ (40%)	DOTA CB-TE2A	PET, Radiotherapy	171, 172
⁶⁷ Ga	3.3 d	EC (100%)	NOTA	SPECT	173, 174
⁶⁸ Ga	68 min	β ⁺ (90%) EC (10%)	DOTA, NOTA	PET	175
⁸⁶ Y	14.7 h	β ⁺ (33%)	DOTA	PET, Radiation dosimetry	176, 177
⁹⁰ Y	64.1 h	β ⁻ (100%)	DOTA	Radioimmunotherapy	178-180
¹¹¹ In	67.9 h	EC (100%)	DOTA	SPECT, Radiotherapy	181-183
¹²³ I	13 h	EC (87%) γ (85%)		SPECT	184
¹²⁴ I	4.2 d	β ⁺ (22%) γ (78%)	Tyrosine residue or Bolton-Hunter moiety	PET	185
¹²⁵ I	59 d	γ (100%)		SPECT	186
¹³¹ I	8.0 d	β ⁻ (89%) γ (81%)		SPECT, Radiotherapy	187
¹⁷⁷ Lu	6.7 d	β ⁻ (100%) γ (27%)	DOTA	SPECT, Radiotherapy	179
¹⁸⁶ Re	3.8 d	β ⁻ (93.1%) EC (6.9%)	HYNIC	SPECT, Radiotherapy	188-190
¹⁸⁸ Re	16.9 h	β ⁻ (100%) γ (15%)	HYNIC	SPECT, Radiotherapy	190-193

EC: electron capture; DOTA: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; NOTA: 1,4,7-triazacyclononane-1,4,7-triacetic acid; CB-TE2A: 2,2'-(1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane-4,11-diyl)diacetic acid; HYNIC: 6-hydrazinonicotinamide

Table 2

Functional components with proven potential for the design and construction of prostate cancer theranostics.

Application	Type or module	Functionalities	References
Active Targeting	Peptide	RGD peptide (cRGDyk)	141, 142
		J591/MLN591	115, 119
		7E11-C5.3	110, 112, 115
	PSMA-targeted Antibody	E6	111
		J415	115
		A10 aptamer	129
	PSMA-targeted Aptamer	A10-3.2 aptamer	130
Passive Targeting	EPR Effect	Carrier's size	73, 74, 100, 101
Diagnostics	MRI	Gadolinium (Gd), SPIO	145-147, 149
	CT	Tri-iodophthalamide	154
	Optical Imaging	Near-infrared fluorophore (Cy5.5 and Rhodamine)	168
	PET or SPECT	PET: ^{64}Cu , ^{68}Ga , ^{86}Y	195-197
		SPECT: ^{111}In , ^{67}Ga , ^{177}Lu , $^{186/188}\text{Re}$, $^{123/125/131}\text{I}$	188, 241-247, 250-252
Therapeutics	Chemotherapy Drug	Curcumin	202-205
		Genistein	206
		Cisplatin	208, 253
		Paclitaxel (PTX)	73, 74, 208, 214, 253
		Resveratrol	207
		Doxorubicin (DOX)	129
		Camptothecins (CPT)	209, 210
		Methotrexate (MTX)	62, 211
	Gene Therapy	Suicide gene (HSK-TK/Cx43)	218
		Small interfering RNAs	224
		miR-15a and miR-16-1	130, 224
	Immunotherapy	Rituximab (anti-CD20)	230

Application	Type or module	Functionalities	References
		Trastuzumab (anti-erbB2)	230, 231
		hMAb425 (anti-EGFR antibody)	231
	Radiotherapy	^{90}Y , ^{64}Cu , ^{177}Lu , $^{186/188}\text{Re}$, ^{131}I	188, 235-237, 241-247