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Fabrication of Dendrimers Toward Biological Application

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I. Introduction	102
II. Are Dendrimers Attractive in Biomedical Sciences?	103
A. In Vitro Cytotoxicity of Dendrimers	106
B. In Vivo Toxicity of Dendrimers	107
C. How to Solve the Problems of Biomedical Toxicity	108
III. Drug Delivery Systems of Dendrimers	110
A. Load of Drugs by Physical Doping in Dendrimers	110
B. Release of Drugs Loaded by Physical Doping in Dendrimers	113
C. Delivery of Drugs Loaded by Chemical Binding in Dendrimers	114
IV. Targeting Delivery of Dendrimers	116
A. Folic Acid Dendrimers for Cancer Targeting	117
B. Antibody-Dendrimer for Cancer Targeting	117
C. Glycodendrimer for Cancer Targeting	118
V. Dendrimers for Therapy	119
A. Dendrimers for Boron–Neutron Therapy	119
B. Dendrimers for Photodynamic Therapy	120
C. Dendrimers for Photothermal Therapy	122
D. Dendrimers for Gene Therapy	122
VI. Dendrimers as Imaging Agents for Inspection	126
A. Molecular Probes	126
B. Magnetic Resonance Imaging	128
C. X-ray Imaging and CT	129
D. Optical Fluorescence Imaging	131
VII. Conclusions	132
References	132

Dendrimers consisting of inner core and peripheral shell are well-designed branching architectures with abundant terminal groups. Therefore, the high level of control over dendritic architectures makes dendrimers ideal carriers in biomedical application. In addition, the toxicity of dendrimers mainly comes from the high cationic charge density in the periphery, where charges interact with biological cell membrane and then result in membrane disruption. Two strategies have been utilized to minimize the toxicity of dendrimers: first, selecting neutral or anionic biocompatible dendrimers, and second, masking of peripheral charge by chemical modification. Then the focus of this chapter shifts to biomedical application of biocompatible dendrimers, including drug delivery systems of dendrimers, targeting delivery by dendrimers, and applications of dendrimers for therapy and as imaging agents for inspection.

I. Introduction

Dendrimers are globular, highly branched macromolecules possessing a well-defined interior region and a large number of end groups. The unique structural features of dendrimers is that there are a number of spacers whose ends combine with a high degree of branching which leads to a variety of new physical properties, different from traditional linear polymers. Dendrimers consist of a core, several branch points, and outer surface moieties, as shown in Fig. 1. The size of dendrimers is defined by generations, each of which corresponds to the layer of branching. Dendrimers are generally synthesized by a sequential repeat of reaction steps and each additional step derives the higher-generation dendrimer. Each created layer (generation) accompanies the doubling of the surface end groups for the two-functional branch and the remarkable increase of the molecular weight.

The ultimate goal of nanotechnology is the handling of technology at the nanoscale (at the range of 1–100 nm), that is, the handling of the inorganic particles, macromolecules, and supramolecules. Especially, nanobiotechnology



FIG. 1. Schematic representation of a second-generation dendrimer with two-functional branch.

provides solutions for transformation of biosystems and offers a nanolevel control of techniques and processes on human life. The development of supramolecular nanostructures with well-defined particle size and shape are of prominent interest in the biological utilization such as delivery of pharmaceutical drugs and imaging agents or gene transfection. In general, architectures utilized as carriers in drug delivery should be in nanometer range and uniform in size to possess their ability for passing through cell membranes and to reduce the risk of undesired clearance from macrophage during blood circulation.

Dendrimers, one of such organic nanoparticles, provide a number of advantages compared to other polymeric nanoarchitectures which have been used for biological purposes. The structural regularity of dendrimers has motivated numerous studies with the aim of biomedical applications; for example, the enhancement of molecular effect or the creation of extremely high local concentration of drug, gene transfection, molecular label, or probe moiety. Moreover, the nanometer size of dendrimers and their narrow polydispersity can allow easier passage across biological barriers such as an extravasation through vascular endothelial tissue. The versatile poly(amido amine) (PAMAM) dendrimer and poly(propylenemine) (PPI) dendrimer as shown in Fig. 2 have been most widely investigated for these applications. The first- to tenth-generation PAMAM dendrimers with terminal groups such as -COOH, $-NH_2$, or -OH have been synthesized and then their size varied from 1.1 to 12.4 nm. In this chapter, the fabrication of dendrimers as biocompatible nanoparticles toward medical application is reviewed. After commenting on the overcoming of toxicity, the application to drug, and targeting delivery systems, therapy and inspection by imaging agents will be introduced.

II. Are Dendrimers Attractive in Biomedical Sciences?

Dendrimers have a three-dimensional structure of nanometer size without distribution of molecular weight. The unique characteristic properties of dendrimers are often compared with the globular shape of proteins and make this class of synthetic macromolecules suitable for the mimicry of proteins. In addition, dendrimers are on average less compact than proteins. The interior of dendrimers is not packed as efficiently as the typical proteins, and dendrimers possess a substantially large number of terminal functional groups superior to proteins of corresponding molecular weight.

It must be mentioned that the monodispersity is one of the important factors to launch dendrimers to the forefront of biomedical research. Monodispersity is a key, and it allows the investigation of explicit correlation between dendritic structure and biological system. By knowing the exact composition of



FIG. 2. Second-generation PAMAM dendrimer and PPI dendrimer.

macromolecules, scientists can determine the biological activity related to their specific configuration. This is a powerful tool for drug and medical systems. However, it must be noticed that dendrimers of high generation have some

104

defects in the defined dendrimer structure because of a too crowded situation of peripheral functional moieties. Moreover, most dendrimers could be mounted with more than one type of ligand, such as targeting moiety, imaging moiety, and therapeutic moiety, all suitable to be conjugated in the periphery of one dendrimer.

Another important feature is the multivalent behavior of dendrimers. Multivalency leads the strong activity, as shown in Fig. 3, compared to the corresponding monomeric interaction. This synergistic enhancement of binding affinity from a monomeric to multimeric system could be ascribed as cluster effect or dendritic effect. The dendritic effect comes into working when simultaneous existence of N-binding sites in a ligand molecule results in a synergistic increase in affinity with a maximum binding affinity of N multiplication. Besides, the cluster effect can be usually observed for carbohydrate–protein receptors in a natural system, known as glycoside cluster effect.¹ Another important issue is that multivalency can also increase the specificity of a given interaction.² A factor that plays a role in the binding of ligands in a dendrimer is obviously the geometry of multivalently existing ligands and the flexibility of their attachment. Polyvalency of dendrimers orients bare reactive groups on the nanostructural exterior of dendrimers.



FIG. 3. Mutivalent behavior of a dendrimer on a cell membrane.

A. In Vitro Cytotoxicity of Dendrimers

A major concern on the adoption of a new class of nanomaterials in biomedical systems is toward the biocompatibility of these materials. In order to be usable as drug delivery, gene delivery, and imaging agents, dendrimers have to be nontoxic and nonimmunogenic. The cytotoxicity of dendrimers has been evaluated *in vitro* and even *in vivo*. Cytotoxicity of dendrimers is dependent on their chemical structure but is most strongly influenced by the nature of their terminal groups. Depending on the modification of terminal groups on dendrimers, dendrimers carry a positive, neutral, or negative charge on their periphery. For example, among melamine-based dendrimers carrying amine, guanidine, carboxylate, sulfonate, phosphonate, or poly(ethylene glycol) (PEG) on the periphery, cationic dendrimers.³ The positive charges on the dendrimer tend to destabilize cell membrane, cause cell lysis, and interact with blood components.^{4–7}

The mechanism of membrane damage induced by a cationic PAMAM dendrimer was investigated by using 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine liposome and human nasopharynx carcinoma (KB) and rat embryo fibroblast (Rat2) cells in culture.^{5,8} Techniques such as atomic force microscopy, fluorescence microscopy, and confocal microscopy were used to visualize damage and release of enzyme lactate dehydrogenase to explore leak of cell membrane. Further, the cytotoxicity was found to be generation-dependent, that is, the higher generation of dendrimers provided stronger toxicity. The reason might be attributed to the higher-generation dendrimers attaining higher surface coverage on cells and liposome, which might induce cell death or membrane leakage. The mechanism of the interaction of dendrimers with the cell membrane is shown in Fig. 4.

PAMAM dendrimers with amino groups revealed the significant cytotoxicity on human intestinal adenocarcinoma (Caco-2) cells.^{9,10} A generation dependency of amino-terminated PAMAM dendrimers was observed on the hemolytic effect with blood cells in a solution.⁷ However, the related study has shown that amino-terminated PAMAM dendrimers exhibit lower toxicity than flexible linear polymers with amine groups. This should be ascribed to the low adherence of rigid globular dendrimers to the cell surface. The degree of substitution of amine as well as the type of amine functionality is also important, so that the primary amine is more toxic than the second or tertiary amine.¹¹ Similar cytotoxicity and hemolytic effects were found for amineterminated PPI dendrimers, involving the increase of both effects depending on generation.¹² The cytotoxicity of dendrimers having terminal groups such as guanidine, carboxylate, sulphonate, or phosphonate besides amine is also reported.³

106



FIG. 4. Schematic representation of the interaction between dendrimer and cell membrane.

B. In Vivo Toxicity of Dendrimers

Studies of *in vivo* toxicity basically prove the safety of any materials on biomedical application. PAMAM dendrimers have been injected into mice to evaluate the *in vivo* cytotoxicity. Results indicated that nontoxicity in mice was obtained for PAMAM dendrimers with both unmodified and modified amino-terminal groups.^{13,14} *In vitro* and *in vivo* cytotoxicities of polyester dendrimers have been investigated.¹⁵ Inhibition of cell growth was at the concentration of 40 mg/ml but no cell death occurred at the same concentration. Upon injection into mice, no acute or chronic toxicity was observed.

Recent reports suggest that higher-generation dendrimers exhibit greater hemolytic toxicity, which can be ascribed to the greater overall cationic charge. The cationic terminal groups of dendrimers interact with red blood cells. The polycationic nature of dendrimers also leads to hemolysis^{16–18} and might influence hematological parameters attributing to the polycationic nature of unmodified dendrimers. The effect of cationic dendrimers, such as aminoterminated PAMAM and PPI dendrimers, on different blood components including white blood corpuscles (WBCs), red blood corpuscles (RBCs), hemoglobin (Hb), hematocrit (HCT), and mean corpuscular hemoglobin (MCH), could be determined by using a particle counter.¹⁹ Significant decreases in contents of RBC, Hb, and MCH, a substantial increase in the count of WBCs, and a considerable difference of HCT values between the control and cationic dendrimers indicated that cationic dendrimers exhibit impairments in the hematological component. Some reports relating to the immunogenicity of dendrimers revealed no or only weak immunogenicity in mice treated with cationic dendrimers. In addition, from the investigation of the immunogenicity of peptide dendrimers in Bulb/C (a strain of inbred white mice that is readily developed for experimental myelomatosis) mice using the enzyme-linked immunosorbent assay for monitoring of an antibody, it has been observed that the multiple peptide dendrimers are unable to cause any detectable humoral immune response. This means that the dendrimers could be regarded as "pseudo-native" for the host immune system.

C. How to Solve the Problems of Biomedical Toxicity

There are two general ways to reduce the cytotoxicity and hemolytic toxicity of dendrimers. The first approach is the embedding of the biodegradable moieties in the interior of dendrimers. Monomers, which may be transferred to metabolic products via various biological pathways, are selected in the synthesis of dendrimers and hence they lead to the synthesis of biodegradable dendrimers. Monomers such as lactic acid, glycerol, succinic acid, and PEG have been utilized as good biocompatible and biodegradable component materials for the synthesis of dendrimers.^{20–22} Another kind of biodegradable dendrimers was composed of different types of amino acids such as lysine, arginine, etc., and thus produced dendrimers promised to have drug carrier properties.^{23–25} Peptide dendrimers with oxime, hydrazone, or thiazolidine linkage as a building block and a selective ligation between an aldehyde and a weak base were also synthesized.²⁶

The second approach is the modification of the terminal amine/cationic groups of dendrimers with natural or anionic moiety. It is well documented that decreasing the surface charge of amine-terminated dendrimers toward neutral reduces their toxicity. The presence of multiple terminal sites makes possible the attachment of the moieties with various functionalities through the covalent or noncovalent bonding on the periphery of the dendrimers. In addition to the proper reduction of the toxicity of dendrimers, the functionalization also imparts some other properties, which are beneficial for their biological application including improvements of drug control and therapeutic potential.^{18,27-30} It has been proven that hydrophilic PEG chains can enhance the loading efficiency of hydrophilic drugs and cause the stable enclosure of drugs within the dendrimers. In addition, PEG-modified dendrimers could heighten the circulation efficiency in blood, which allows the drug carrier by dendrimers to move toward the targeting specific tissue. Therefore, the PEGylation of dendrimers can effectively reinforce the therapeutic potentials of dendrimers alone.

108

It has been reported that the cytotoxicity related to amine-terminated PAMAM dendrimers for cancer therapy could be minimized by the amidation of terminal amine groups.³¹ Additionally, the amidation of amines in dendrimers leads to a 10-fold reduction in cytotoxicity, while the ability of the cell transmission remains.³² The partial amidation was effective in reducing the cytotoxicity, whereas the complete amidation of terminal amine groups in dendrimers evoked the absence of the cytotoxicity. The number of free amine groups was in linear relation to the cytotoxicity. It was also revealed that the toxicity of terminal-modified PAMAM dendrimers was similar to that of unmodified dendrimers.³²

The modification of dendrimers with chemically inert PEGs or fatty acids is the other way to reduce the toxicity of cationic dendrimers.^{6,9,10} PEGylation of dendrimers not only reduces the toxicity but also improves the drug loading and reduces the drug leakage.³⁰ Other purposes of PEGylation of dendrimers are improvement of biodistribution and pharmacokinetics, increase in solubility of dendrimers, sustained and controlled drug delivery, better transfection efficiency, and tumor localization.^{18,29,33} Additionally, immunogenicity is one of the crucial *in vivo* biological properties of dendrimers. A lot of research revealed that the scarce or slight immunogenicity occurred in dendrimer materials. Other studies also indicated that the PEGylation of dendrimers resulted in relatively low immunogenicity, when compared with non-PEGylated ones. It should be noted that the PEGylated drug-loaded systems have a number of advantages including the retention of bioactivity and bioenvironmental protection. As a general rule, PEG with high molecular weight has been widely used as a drug carrier to improve the targeting and therapeutic efficacy.

Star dendrimers as drug carriers have been synthesized by combining dendrimers on the terminals of three-arm star PEGs.³⁴ The goal of this synthesis was a preparation of long-lived dendrimers for circulating in vivo to allow them to accumulate on the targeting tissue. In the design of drug carriers, the PEG polymer chains could prevent the recognition of macrophages and white cells during their circulation in the blood. Therefore, PEG-modified drug carriers have the long-lived circulating behavior when compared with unmodified ones. Further investigation has been reported to evaluate the biodistribution of a sixth-generation lysine dendrimer and two PEGylated derivatives in both normal and tumor-bearing mice.¹⁵ The intact poly(lysine) dendrimer underwent the rapid clearance from the blood stream and the nonspecific accumulation in the liver and kidney. In contrast, the PEGylated poly(lysine) dendrimer with high-degree modification was accumulated effectively in the tumor tissue. This success of the effect suggested that the PEGylated poly(lysine) dendrimers should be useful materials for the tumor-targeting drug carrier.¹⁵

110

The toxicity of dendrimers could be related to the interaction of terminal cationic charge of dendrimers with negatively charged biological membranes *in vivo*. Such an interaction of dendrimers with biological membranes results in membrane disruption via nanohole formation, membrane thinning, and erosion. To minimize this toxicity, two strategies have been utilized: first, designing and synthesizing of biocompatible dendrimers, and second, masking of peripheral charges of dendrimers by surface engineering. In order to design an adequate dendrimer for biomedical application, one has to pay attention and realize what causes the toxicity of the dendrimer and how to reduce it.

III. Drug Delivery Systems of Dendrimers

Recently, developing nanotechnology-based efficient drug delivery systems for small molecule, protein, and DNA has attracted a great deal of attention. Dendrimers, unlike traditional polymers, can be obtained at the precise molecular weight even at high generation, which can provide reproducible pharmacokinetic results. This feature makes dendrimers an ideal candidate for drug delivery applications. In dendrimer-based delivery, drugs could be noncovalently encapsulated in the interior of dendrimers by the physical association or covalently conjugated to form prodrugs, as shown in Fig. 5.

A. Load of Drugs by Physical Doping in Dendrimers

Dendrimers consisting of a hydrophobic interior and a polar periphery may be referred to as monomolecular micelles without the limitation of the critical micelle concentration. Several therapeutic agents are examined as guest molecules for drug delivery. Hydrophobic anticancer drugs such as camptothecin are proven to damage DNA and lead to cell death. However, the extremely low water solubility of camptothecin limits its therapeutic efficiency. In order to solve this problem, the hydrophobic camptothecin has been utilized by encapsulating it in liposome, micelles, nanoparticles, and dendrimers.³⁵ A biocompatible polyether dendrimer has been modified to comprise glycerol and succinic acid to conjugate with 10-hydroxyl camptothecin.¹⁸ The cytotoxicity of the dendrimer-drug complex showed a low IC₅₀ (nmol/L) (half-maximal (50 %) inhibitory concentration) value; moreover, the measurement in human breast adenocarcinoma cells showed a 16-fold increase on cellular uptake and an increase on drug retention. Otherwise, dendrimers based on 1,3,5-triazine have been also utilized for the encapsulation of 10-hydroxyl camptothecin.³⁶ The building block, triazine, attracts a lot of attention due to not only its low cost but also the easy displacement of three chlorine atoms by amines to



FIG. 5. Schematic representation of a functional dendrimer adapted as drug carrier.

generate mono-, di-, and tri-substitute of 1,3,5-triazine. A second-generation triazine dendrimer has been scaled to kilogram quantities, which is an important issue in pharmaceutical applications.³⁷

Another example of loading of drugs within dendrimers is the complexation of small molecular drugs. The small acidic molecules such as benzoate could bind within the interior of the PAMAM dendrimer via ion-pairing with protonated tertiary amine.^{38,39} When benzoate is protonated in acidic condition, this nonionic guest suffers release and precipitation. Dendrimers often encapsulate small organic molecules (drugs) into the void space in the dendrimer's interior, while large guest molecules prefer to absorb onto the dendrimer periphery. Ibuprofen, an anti-inflammatory drug, was complexated and encapsulated into third- and fourth-generation PAMAM dendrimers.⁴⁰ A third-generation PAMAM dendrimer binds 32 molecules of ibuprofen, while a fourth-generation dendrimer with 64 terminal amine groups binds 78 molecules of ibuprofen.^{41,42} It was found that ibuprofen is not only complexated with the terminal amine groups through electrostatic interaction but also encapsulated in the interior of larger dendrimers. In this study, ibuprofen was successfully transported to A549 human lung epithelial carcinoma cells by PAMAM dendrimers. The anti-inflammatory effect of the ibuprofen-complexated dendrimer system revealed more rapid suppression than the pure drug.

The electrostatic binding between the drug and the dendrimer was dependent on concentration and generation.⁴³ The presence of a PAMAM dendrimer enhanced the transdermal delivery of ketoprofen and increased 2.73-fold the bioavailability of a free drug. The solubility of ketoprofen in a PAMAM dendrimer increased linearly with the concentration of dendrimer under the fixed pH condition. The solubility of ketoprofen was highest at pH 6 and lowest at pH 3.⁴² This result indicates that the drug would not be fully ionized at low pH and strongly interacted with a dendrimer. *In vivo* study of ketoprofen–PAMAM dendrimer complexes indicated the prolonged pharmacodynamic behavior of ketoprofen. This suggests that PAMAM dendrimers can act as potential drug carriers of ketoprofen with a sustained release behavior.⁴⁴

One of the methods for physical drug loading is the increased density of terminal groups in higher-generation dendrimers. At a particular generation level, the terminal groups will reach the compact packing limitation, which is called "de Gennes's dense packing."^{45,46} This situation will seal the interior void and prohibit drug penetration. However, the limitation of peripheral moieties depends on the strength of the intramolecular interaction between adjacent moieties, the peripheral shell thickness, and the environmental condition of dendrimer solutions such as temperature, pH, and polarity. Moreover, the dynamics of dendrimer chains is also one of the important factors.^{47,48} These characteristics can promote the encapsulation and release of drugs in and from dendrimers, respectively.

The maximum amount of drug loading was directly related to the shape and size of internal cavities of dendrimers. Large and small molecules could be simultaneously entrapped within PPI dendrimers, which generally contain 4 large and 12 small cavities. PPI dendrimers could be opened under controlled conditions to release either some or all loaded molecules.^{46,49} In a thermodynamic aspect, free drugs can be distinguished from this physical load as a complex with finite energy barriers.⁵⁰ This finite energy has a relation with the inlet and outlet of drugs in dendrimer cavities. A hydrophobic drug would be expected to be associated with the hydrophobic interior of a dendrimer. Further, the hydrophobic property of a drug should isolate itself from the polar periphery of a dendrimer and the polar media to keep the minimum contact with polar environments. Generally, when the drug molecule is significantly larger than the dendrimer cavity dimension, the complexation between drug and dendrimer will not occur.

In order to study the effect of ethylene glycol dendrimer on solubility enhancement of paclitaxel, poly[oligo(ethylene glycol) methacrylate] (poly (OEGM)), star-shaped poly(OEGM), and polyglycerol dendrimers (G3, G4, and G5) have been synthesized and evaluated for their drug-loading properties.⁵¹ Poly(OEGM) increased the solubility of paclitaxel, but a significantly enhanced effect was observed in the star poly(POEGM) and polyglycerol dendrimers. The solubility of paclitaxel in water in the presence (10 wt%) of a star-shaped poly(OEGM) with five arms and polyglycerol dendrimers (G3, G4, G5) was 130-, 270-, 379-, and 434-fold greater than the solubility of paclitaxel in water. This result indicates that the solubility of paclitaxel is not enhanced by a molecular weight of PEG. It was strongly suggested that the high density of the ethylene glycol unit in the dendritic structure contributes to the physical loading of the hydrophobic paclitaxel drug.

B. Release of Drugs Loaded by Physical Doping in Dendrimers

Response to external stimuli is one of the most fundamental properties of the biological system. Indeed, bio-organisms exhibit very high sensitivity to their external environment, changing their shapes or releasing the chemicals from them as a response. Generally, the structure of dendrimers consists of three main components: (1) the core affects the three-dimensional shape of dendrimers, for example, spherical or cylindrical shape; (2) the interior has an influence on the guest–host properties of dendrimers; and (3) the periphery of dendrimers can be further polymerized or modified with functional groups. Dendrimers at low generation are usually flexible and open, while dendrimers at high generation are usually dense with three-dimensional shapes. The conformation of dendrimers could be affected by the ionic strength and pH in aqueous solutions.

The mechanism of drug release can be represented based on molecular conformation change in different pHs and ionic strengths. Conformation of amine-terminated PAMAM and PPI molecules is globular and compact at high pH than pK_a (=9.2).⁵² At pH > 9, the folding of terminal groups occurs as a consequence of hydrogen bonding of the interior amide and/or tertiary amine with the primary amine, which results in a dense interior. The extended conformation dominates at lower pH than pK_a (= 6.7) because of the electrostatic repulsions of protonated ternary amines in the interior. In the case of carboxyl acid-terminated dendrimers, at lower pH than pK_b (=4.2),⁵³ the dendrimer interior has the extended conformation due to the electrostatic repulsion of protonated tertiary amines. While at neutral pH (4.2 < pH < 6.7), the slight folding of terminals takes place as a result of attractive Coulomb's interaction between negatively charged terminal carboxyl groups and positively charged interior tertiary amines, at high pH (6.7<), the electrostatic repulsion between negatively charged terminal carboxyl groups generates an extended conformation due to highly expanded peripheral area.

114

When a PAMAM dendrimer physically loads a guest drug, the drug could be retained within the dendritic void by an electrostatic or hydrophobic interaction with the interior of the dendrimer. Then pH- and temperature-responsive drug carriers are known to be successful drug-releasing systems. A poly(*N*-isopropy-lacrylamide)-conjugated star-shaped dendrimer has been synthesized.⁵⁴ The aqueous solution of this dendrimer was thermo-sensitive, since the dendrimer achieved a lower critical solution temperature (LCST) around 32 °C. Moreover, the addition of methacrylic acid besides NIPAAM not only induces the shift of the LCST but also makes the dendrimer materials responsive to both temperature and pH.

C. Delivery of Drugs Loaded by Chemical Binding in Dendrimers

Dendrimer-drug conjugates generally consist of drugs covalently attached to the periphery of the dendrimer. This type of drug carrier offers different advantages over physically encapsulated drug carrier systems. Drugs can be conjugated with each dendrimer molecule, and the release of these therapeutic molecules could be controlled by the character of linkage. Drug molecule can also become an integral part of the dendritic carrier that is released through certain triggering events at the desired location, for example, a tumor site. The main advantage of these methods is that the drug-dendrimer conjugate diffuses slowly, different from the case where the free drug is absorbed on the specific interface. Therefore, cell targeting and controlled delivery are allowed. An additional benefit of this prodrug is in reproducible pharmacokinetics and pharmacodynamics. The covalent bond between dendrimer and drug also provides a stable structure for the internal encapsulation of drugs, which is less contributed by thermodynamics and physical factors.

The pharmacodynamic behaviors of two types of amide-bonded PAMAM dendrimer–methotrexate (MTX) drug carriers were evaluated.⁵⁵ One of the amide bonds was prepared by coupling a carboxyl-terminated G2.5 PAMAM dendrimer via two amine groups with an aromatic ring of MTX (drug carrier A). Another was synthesized by coupling an amine-terminated G3 PAMAM dendrimer with a γ -carboxyl group of MTX (drug carrier B). A drug carrier A with an MTX payload of 2.8 exhibited a significant anticancer activity toward MTX-resistance cells. However, the drug carrier B with an MTX payload of 22.4 revealed no cytotoxicity on the same cells. A chemical linkage of MTX with dendrimer is shown in Fig. 6. This result can be explained by the different ionic characters of these drug carriers, which results in the different residence time in the lysosomes. It was indicated that in the lysosome by the positively charged dendrimer occurs with an increase in pH and eventually the disruption of the



FIG. 6. Ester and amide linkages by MTX chemotherapeutic drug.

lysosome. As a result, the conjugate linkage reduces the interaction with protease and retards the breaking of drug linkage. Therefore, the different behaviors of the intracellular drug release in drug carrier A and B lead to different cytotoxicities against the MTX-resistance cells.

A remarkable asymmetric architecture, where half of the dendritic drug carrier was modified with doxorubicin (DOX) and the other half with PEG, has been reported.⁵⁶ A pH-sensitive linkage between the drug and dendrimer was designed to release the drug once it is in cells. In the cell culture, the dendrimer–DOX prodrug was less than one-tenth toxic than free DOX on C-26 colon carcinoma cells after culturing for 72 h. Administration of DOX-conjugated dendrimer over 2 months caused complete tumor regression, while no cures were observed for mice treated only by the drug.

Drugs could attach via linkers or spacers to terminal groups of the dendrimer. Usually, ester or amide bonds are employed, since they can be hydrolyzed inside the cells by an endosomal or a lysosomal enzyme.^{57,58} The drug release from a conjugate is controlled largely by the nature of a linking bond or a spacer between the drug and the target physiological domain for the intended release. Ester and amide bonds might be cleaved by enzymes or under hydrolytic condition. However, the ester cleavage is generally more facile than amide cleavage for the drug release.

The stability and release properties of both ester and amide bonds between Naproxen and PAMAM dendrimer were reported.^{59,60} The amide bond conjugation withstood 80% the release in human plasma, indicating that the amide link conjugation exhibited stability in plasma. Meanwhile, the ester link conjugation suffered rapid esterase-catalyzed hydrolysis and the half time of cleavage

cutoff was around 51 min, that is, the ester link conjugation releases the drug easily. In another experiment, a hydrazone bond and an amide bond between PEGylate PAMAM dendrimer and adriamycins have been studied with respect to their release properties.⁶¹ The hydrazone bond of adriamycins was remarkably released at pH 5.0 for the endosome, which is comparable to the amide bond. Further, the conjugation by the hydrazone bond was seven times more efficient than by the amide bond.

Regarding the effect of molecular weight and size difference, the release properties of a high generation of dendrimer have been recently studied.⁵⁹ The three-dimensional nanostructure of dendrimers gives rise to sterical enzymatic release, different from the linear polymer system. Therefore, there should be space for the linkers and the specificity for enzymes on the dendrimer periphery. A drug carrier of tetra-peptide, Gly-Leu-Phe-Gly (GLPG), which is enzymatically cut off, has been applied for studying the drug release. For the GLPG peptide bond of ibuprofen by amide conjugation with PAMAM dendrimer, while the hydrolysis of ester conjugation showed a clear pH-dependent rate, the amide bond conjugation was very stable at all buffer pH. The linkage of peptide between drug and PAMAM dendrimer must rely on enzymatic breakage. The insertion of peptide spacer gives an access site of the enzymes in the presence of cathepsin B. As a result, the dendrimer-GLPG-ibuprofen released 40% pay loads of ibuprofen for 2 days, while the ester and amide bonds of ibuprofen remained at 95% conjugation in the dendrimer. Since steric hindrance played an important role in the enzymatic cutoff from the dendrimer conjugation, the appropriate choice of binding could raise a preferable effect on drug delivery.

IV. Targeting Delivery of Dendrimers

Multifunctional dendritic architecture allowed for the conjugation of both drug and targeting moiety such as folic acid, monoclonal antibody, and peptide to the periphery of the dendrimer for promoting the specific drug delivery (Fig. 5). In the field of oncology, the targeting delivery of chemotherapeutics to the tumor significantly reduces the side effect, compared with the nontargeting delivery, which might damage the healthy tissue such as liver, spleen, kidney, and heart with the accumulation of a toxic level of the drug. Nonspecific protease targeting or passive targeting to tumors could be achieved by PEGylation to increase the hydrodynamic radius of the drug carrier, leading to the accumulation of dendrimers in the tumor through the enhanced permeability retention (EPR) effect. The EPR effect caused the neovascular leak and defection with disorganized endothelial cells, which offers the accumulation and the retention of macromolecules in the tumor region. This may increase

117

the effect of the antitumor drug up to 70-fold, when drug delivery systems are injected intravenously.⁶² Specific targeting or active targeting relies on the conjugation of one or more targeting moieties in dendrimers to facilitate the receptor mediate endocytosis against cells.

A. Folic Acid Dendrimers for Cancer Targeting

The binding affinity of a multivalent targeting G5 PAMAM dendrimer containing a different number of folic acid moiety has been explicitly quantified.⁶³ The binding affinity to a folic acid receptor expressing cells increased with numbers of folic acid in the dendrimer up to 5–6 folic acids per dendrimer. However, the rate of internalization was not affected significantly at different numbers of folic acids. It was suggested that aggregates of 5-6 folic acid receptors are prerecognized on the cell membrane. The main discovery in this study indicated that the efficiency of the targeting cell is enhanced by the retention of receptors on the cell but not the rate of endocytosis. Several variations of folic acid-conjugated dendrimers for targeting drug delivery have been investigated.⁶⁴ Folic acid was conjugated on the G5 PAMAM dendrimer with remaining free amines, which were capped with glycidol for neutralization of positive charge on the dendrimer. Results in the drug release profile were compared between encapsulated MTX and covalently bound MTX. Physically loaded MTX showed the rapid release of the free drug (around 75% of initial load) within 2.5 h, while a slower release for chemical bond was only around 5% of the initial load in the same period. In addition, it was demonstrated that folic acid conjugated on the dendrimer was highly specific for KB cells (human carcinoma of the nasopharynx), overexpressing the folic acid receptor. Although both the free drug carrier and the actively targeting dendrimer-based drug carrier showed similar cytotoxicity, the targeting dendrimer lost the targeting effect when the receptor was blocked or underexpressed with free folic acid.

B. Antibody-Dendrimer for Cancer Targeting

Antibody-conjugated delivery systems are the novel strategy for delivery of therapeutic agent to specific cell type. Conjugation of monoclonal antibody to PAMAM dendrimer has been investigated for specific targeting of tumor cells that overexpress specific antigen.⁶⁵ Another targeting moiety J591, an antiprostate specific antigen (PSA), was conjugated on a G5 PAMAM dendrimer and evaluated the cell binding and the internalization ability. The antibody-conjugated dendrimer has been found to bind specifically to PSA positive cells (LNCaP; human prostate cancer cell lines from lymph) but not to PSA negative cells (PC-3; human prostate cancer cell lines from bone). Further, the targeting

efficiency of the antibody-conjugated dendrimer was evaluated from the image of a confocal microscope. The unconjugated dendrimer was not significantly taken in by a cell.

An anti-Her2 G5 PAMAM dendrimer tagged with AlexaFluro was synthesized for targeting HER2 expressing cells.⁶⁶ The conjugation of Anti-Her2 on the dendrimer was applied for the targeting of human growth factor receptor-2 overexpressing breast and ovarian cancer cells. Not only did the conjugation yield the preferable binding and affinity but also the *in vivo* result indicated that it targets the HER2 expressing tumors. The effect of conjugation on the immunoreactivity of antibody has been examined with respect to size and number of conjugation.⁶⁷ An anti-epidermal growth factor receptor antibody hMAb425 was conjugated with PAMAM dendrimers of different sizes containing up to 128 chelating moieties. Results in this study indicated that the number of derivated sites has a crucial effect on immunoreactivity, whereas the size of the dendrimer did not give a significant influence on activity.

C. Glycodendrimer for Cancer Targeting

The biological character of the carbohydrate receptor allows the carbohydrate cluster to be applied to a receptor with biologically meaningful affinity. Carbohydrate in some of the glycoconjugates played a key role in the process of cell-to-cell and cell-to-pathogen adhesion. Therefore, the glycodendrimers have several peripheral groups of carbohydrate residues accessible for multiple binding interactions. To evaluate such constructs, several T-antigens containing glycodendrimer clusters were synthesized for checking relative binding properties. Even though glycodendrimers may be less efficient than glycopolymers on the inhibition effect, the structure of the dendrimers is worthy of notice because of the lack of immunogenicity and the well-defined chemical pattern. Since glycodendrimers are chemically and geometrically well-defined monodispersed macromolecules, they are suitable as a tool for medical and pharmaceutical purposes. Glycodendrimers are a class of dendrimers that incorporate the sugar moiety such as glucose, galactose mannose,⁶⁸ and disaccharide.⁶⁹ A glycosylation of dendrimer was prepared and evaluated along an HeLa cell (cancer cell) line and a nontransformed mouse embryonic fibroblast cell (MEF, healthy cell) line.⁷⁰ While the glycopeptide dendrimers conjugated were not as antiproliferative as colchicine alone, the dendrimers were 20-100 times more effectively inhibiting the proliferation of HeLa cells than MEF cells. However, nonglycosylated dendrimers were 10-fold less selective for HeLa cells.

Chemically and geometrically well-defined T-Ag glycol PAMAM dendrimers with valences of 4, 8, 16, and 32 were synthesized with an amide linkage. Successive bioassays showed strong protein-binding properties, thus demonstrating an excellent cluster effect that is establishing these as a strong candidate for biological and immunochemical applications.⁷¹

V. Dendrimers for Therapy

A. Dendrimers for Boron–Neutron Therapy

Boron–neuron capture therapy (BNCT) is a binary approach to the treatment of cancer. Biologically targeted BNCT treatment is based on the production of radiation inside a tumor using ¹⁰B and thermal neutron. For BNCT to be effective on a tumor, a minimum concentration of ¹⁰B must be around 10–30 µg/g of the tumor. If ¹⁰B can be delivered in sufficient quantities to the tumor tissue, the subsequent irradiation with thermal or epithermal neutrons produces a highly energetic α particle and ⁷Li³⁺ ion that could damage the mitotic potential of the tumor cells.^{72,73} A critical issue for BNCT is the precise localization of a boron therapeutic agent in the tumor. In this perspective, dendrimers are a very fascinating material for using as boron carriers due to their well-defined structure and multivalency as shown in Fig. 7.

In order to reach the above goal, in 1994, the starburst PAMAM dendrimer has been boronated by reacting them with an isocyanatopolyhedral borane $(Na(CH_3)_3NB_{10}H_8NCO)$.⁷⁴ This compound was attached to monoclonal antibodies (IB16-6) to produce an immune conjugate for selective cell targeting. *In vivo* biodistribution studies revealed that this dendrimer–borane agent was localized with strong propensity in the liver and spleen. In subsequent studies, in order to achieve the effective brain tumor targeting, an epidermal growth factor (EGF) was attached to the boronated G4 PAMAM dendrimer, as gliomas express a level of epidermal growth factor receptor. It was found that the boronated G4 dendrimer was initially bound to the cell membrane and then endocytosed, resulting in an accumulation of boron in the cell lysosome, which was determined from the energy-filtered transmission electron microscopy known as electron spectroscopic imaging.⁷⁵ Another monoclonal antibody, cetuximab, which was directed against mutant isoforms of EGF, has been



FIG. 7. An action of a dendrimer as a BNCT carrier.

evaluated as a boron delivery agent for brain tumor. After 24 h of the administration of boronated cetuximab-conjugated dendrimer, the mean boron concentration in rats bearing gliomas was found to be 92.3 ± 23.3 µg/g. In contrast, the accumulation of the nontargeting boronated dendrimer was only $6.7 \pm 3.6 \ \mu g/g$ in tumor. Moreover, the mean survival time of rats injected with a targeting boronated dendrimer was 45 ± 3 days as compared to 25 ± 3 days for nontargeting ones. These results demonstrated the efficacy of the boronated cetuximab on BNCT of an intracerebral glioma, and this examination can be regarded as a model for future studies.^{76,77}

B. Dendrimers for Photodynamic Therapy

In photodynamic therapy (PDT), the drug becomes toxic upon *in situ* irradiation to generate the small amount of toxic singlet oxygen or radical oxygen, which has strong physiologically damaging effects. The photosensitive drug should be relatively nontoxic under nonirradiative conditions. According to the principle of light irradiation, the photosensitive drug is excited to the singlet state and then transformed to the long-lived triplet state. The triplet state of the photosensitive drug can transfer the excited energy to oxygen molecules to generate reactive oxidative species (ROS). Therefore, the selective photoirradiation of the target tissue could possibly minimize damage to the healthy tissue.

Since the initial approval of porphyrin as a photosensitizer for the cure of bladder cancer, the use of PDT for the treatment of cancer as well as the non-neoplastic lesion has increased dramatically, following advances in laser light and photosensitizer.⁷⁸ Until now, a number of these photosensitizers have gained regulatory authorization and some photosensitizers are under clinical evaluation.⁷⁹ Most of the photosensitizers show less solubility in water and poor selectivity for tumors. It is clear from the discussion above that dendrimers could be used for overcoming less solubility, nontargeting, toxicity, and other problems, since they are impeditive in the bioactivity of photosensitizers. Therefore, the dendritic carrier can be effectively manipulated to improve PDT.

Polyamide-based dendrimers with 5-aminolevulinic acid (ALA) have been prepared, and their activity has been evaluated on the photodynamic examination.⁸⁰ Protoporphyrin IX (PPIX) was formed from the substrate of ALA via the heme synthesis pathway in cells. Formation of PPIX in the cells can be used for tumor detection or for PDT. On the investigation of G0-ALA dendron with three ALA groups at the periphery, results revealed that similar PDT efficiency was obtained for G0-ALA when compared with free ALA. For G0-ALA in the cells, the ALA molecules are first cleaved from the dendron and then go within the cells through the heme synthesis pathway.^{81,82} Lipophilicity and esterase (hydrolase enzyme) accessibility were also evaluated through a series of G0-ALA dendrons containing different focal points and linkers. Partition

120

coefficient was calculated for the G0-ALA dendron with a nitro core and a propyl linker (3H-ALA), which was found to be the most lipophilic.⁸³ Analysis of the dendron structures indicated that both lipophilicity and accessibility to the ALA ester linkage might lead to the comparatively higher success of 3H-ALA.

A larger-generation (second-generation) ALA-based dendrimer with terminals of 18 ALAs (G2-18-ALA) was synthesized from a tripodent aromatic core by an ester linkage and its ability to deliver a high payload of ALA molecules was examined.⁸⁴ At low concentration, G2-18-ALA was superior to free ALA on the formation of PpIX. G2-18-ALA with propionamido linker was demonstrated to have slightly high efficacy, when compared with free ALA. This indicates that the existence of propionamido linker in the G2-18-ALA may raise a small steric advantage to make the ALA residue more accessible to enzymatic cleavage. In addition, G2-18ALA was found to be internalized via pinocytosis, while the G0-ALA goes through the cells by its active transport and passive diffusion.

Most photosensitizers are poorly soluble in water because of their large π -conjugation and hydrophobic characteristics. Therefore, photosensitizers easily form aggregates in water, which could result in the reduced ROS activity due to the self-quenching of the excited state.⁸⁵ Poly(benzyl ether) dendrimer with photosensitizer in the core has been synthesized.⁸⁶ The substitution of large dendritic wedges offered the isolation effect for photosensitizer and avoided the aggregation of photosensiziter as shown in Fig. 8. Cell viability of a dendrimer-photosensitizer system was evaluated for Lewis lung carcinoma after photoirradiation. The result showed that a dendrimer–porphyrin system is 10–100 times more photocytotoxic than a conventional photosensitizer PPIX. Polyion-complexified micelles could be prepared from ionic dendrimer–porphyrin and polyelectrolyte with counter ions for the examination of *in vitro* and *in vivo* PDT efficiency. High stability in the increasing ionic strength and further enhancement in photocytotoxicity were obtained in the



FIG. 8. Aspect of a drug in the core of a dendrimer for prevention of drug aggregation (left) in comparison with a free drug without a dendrimer (right).

polyion-complexified micelles. In addition, the large hydrodynamic volume of micelles was expected to result in high localization into tumor tissue due to the enhanced permeation and retention effects.⁸⁷

C. Dendrimers for Photothermal Therapy

Gold-based nanoparticles have been developed through different routes for a photo-induced therapy, called photothermal therapy. Photothermal therapy is unlike PDT, which could work in the target tissue or cells without oxygen. Gold nanoparticles strongly absorb light in the visible and near-infrared regions. Especially, near-infrared light can penetrate relatively deep into biological tissue. Therefore, gold nanoparticles can generate the lethal dose of heat at the site of the tumor.⁸⁸ Amine-terminated G5 PAMAM dendrimerencapsulated gold nanoparticles were prepared and conjugated with fluorescein and folic acid for active drug carrier targeting.⁸⁹ The dendrimers displayed the specific binding with KB cells *in vitro* and were located in lysosomes within 2 h. The utility of these nanoparticles for target hyperthermia therapy and the *in vivo* performance research are still in progress.

Photothermal properties of PEGylated and non-PEGylated G4 PAMAM dendrimers, where gold nanoparticles are entrapped, have been investigated.⁹⁰ The gold nanoparticles were prepared by the reduction of HAuCl₄ in a dendrimer solution. The light irradiation produced an increase in temperature of the aqueous solution of the gold-encapsulated PEGylated dendrimer, indicating that gold particles retain photothermal properties even if they are encapsulated in a dendrimer. In addition, the gold-encapsulated PEGylated dendrimer was superior on photostability to the non-PEGylated one. *In vivo* results showed that the gold-encapsulated non-PEGylated dendrimer decreased excitation intensity within 3 days, but the intensity of the gold-encapsulated PEGylated dendrimer could get additional beneficial contribution from the dendrimer, such as acting as a carrier to load chemotherapeutic drugs for double therapy.

D. Dendrimers for Gene Therapy

Gene therapy is emerging as a potential strategy for the treatment of genetic disease, cancer, cardiovascular disease, and infection disease. Delivery of plasma DNA or virus-based protein expression vector is considered a promising approach for the cure of hereditary disease by substituting defective genes. In case of DNA delivery, the nucleic acid needs to be introduced into the nucleus. However, a 21–23 basepair (bp) long double-stranded small interfering RNA (siRNA) could act in the cytosol, where a single strand of siRNA is integrated into the RNA-induced silencing complex.^{91,92} Although





FIG. 9. Action of cationic dendrimers as gene carriers.

other forms of siRNA such as short hairpin RNA (shRNA), microRNA, and so on are available, gene encoding for shRNA, like plasmid DNA, has to enter the nucleus, which causes the complexity in the gene delivery. Figure 9 represents the action of the dendrimer as a gene carrier for DNA and siRNA.

Free DNA or siRNA can be intravenously injected in the patients but they might be rapidly degraded by serum nucleases. As a result, vectors that can protect and downsize the nucleotides are vital. There are two kinds of vectors that could be used in gene delivery: viral and nonviral vectors.^{93,94} Viral-mediated nucleotide vehicles play an important role in gene therapy but have a risk for safety such as immunogenicity and potential viral recombination. Therefore, more attention has been concentrated on nonviral vectors. Nonviral vectors such as cationic liposomes and polycations have attracted much attention due to their low immune response and safety. However, the low transfection efficiency greatly limits their clinical application. Scientists have been motivated to use polymers as gene-transfer systems for several reasons, including the fact that nucleic acids themselves are polymers with the nature of negative charges and spontaneously form complexes ("polyplexes") with cationic polymers.

The main restrictions for *in vitro* gene delivery can be categorized as follows: (1) complex stability, (2) lysosome escape, and (3) dissociation of gene from complex at target. Genetic materials are usually taken into the cell by the endocytosis pathway. At first it should be located at endosomes and then transported to lysosomes or cytosol. Thus the genetic materials reach endosomes in a mildly acidic condition and then end up in lysosomes, in which they face much lower pH condition and might be degraded by enzymes. Therefore, an important issue for the gene carrier is to escape from endosomes and to deliver it to cytosol, which is an extremely important process for effective gene therapy.

124

One reason for the high efficiency of PPI and PAMAM dendrimers in gene transfer is their stimulus behavior in response to the endosomal environment. These dendrimers are like sponges which can absorb protons. It means that the moderate protonation of nitrogen occurs at acidic pH, but the protonation of nitrogen would also arise with endosomal acidification. Finally, the increased density of positive charges causes an influx of chloride ion and water into the endosome.⁹⁵ In consequence, the endosome bursts due to the elevated osmotic pressure and the membrane-destabilizing effect of positively charged dendrimer (polymer), which is also called the "proton sponge effect." However, it still exhibits a bottleneck which has to be optimized for the delivery of therapeutic nucleic acid in efficient endosomal escape. Adequate amount of PAMAM dendrimer per DNA is preferable as the dendriplex for gene transfer,⁹⁶ where the word dendriplex describes the complex formed from the electrostatic interaction between cationic dendrimers and anionic DNA/RNA. Moreover, the gene transfer of PAMAM dendrimer with high generation is likely superior to that of low generation.^{97–99} The transfection efficiency of the dendrimer shows linear increase with increase of generation up to 6, while the higher generation of PAMAM dendrimer (G > 7) reversely behaves in the transfection efficiency. These results could be ascribed to the augmented toxicity of the dendrimer as the generation increases. Therefore, the development of low generation of dendrimers (G < 4) in the gene carrier has attracted more attention due to the extremely low cytotoxicity.¹⁰⁰

Different strategies have been used to improve the transfection efficiency and the biocompatibility of the dendrimer gene carrier. The enhanced gene transfer activity through the conjugation of α -cyclodextrin (α -CD) with the dendrimer could be ascribed to the disruption on endosome membrane.¹⁰¹ Moreover, α -CD has also been reported as a good candidate for siRNA delivery.^{102,103} The ternary complex of α -CD/PAMAM dendrimer/siRNA induced sequence-specific gene silence without off-target effect. In this system, α -CD was found to have the potent RNA interference effect compared to lipofectamine 2000 (L2000), a commercial agent for siRNA transfection.

To improve the targeting and the membrane penetration of the gene carrier, a cell-penetrating peptide conjugated in the dendrimer was developed: Modifying the amine groups of the dendrimer with arginine moiety could achieve the above goal.^{104–106} Dendriplexes including arginine showed up to two orders of magnitude higher DNA transfection efficiency than dendriplexes without arginine in liver hepatocellular (HepG2), mouse neuroblastoma (Neuro2A), and smooth muscle cells. Gene transfection efficiency of the arginine-conjugated dendrimer could be improved by replacing the ester group in the amide bond. Confocal microscopic images indicated that the ester groups in arginines were cut off via the ester hydrolysis, since the hydroxyl-terminated PAMAM dendrimer did not interact with anionic protein

in the cells. Studies also mentioned that high transfection efficiency could be further obtained by increasing the number of arginine in the periphery of the dendrimer. Di-arginine dendriplex could be found inside the nucleus, while mono-arginine dendriplex was mainly located inside the cytoplasm.

The targeting moiety has to attach onto the periphery of the dendrimer to increase the efficient delivery of the complex to the specific tissue.¹⁰⁷ In vivo targeted hepatic gene delivery was achieved by conjugating G3 PPI dendrimers with a galactose group. After intravenous (i.v.) injection of asialoglycoprotein receptor-targeting dendrimer complex with DNA into CD-1 mice, organs were harvested to check the luciferase expression. It was confirmed from the investigation of specific liver gene delivery that the luciferase expression occurred preferentially in the liver over other organs. The synergetic effect of targeting agents was evaluated from another *in vivo* study using a PPI dendrimer as a delivery agent. The siRNA was complexified with the G5 PPI dendrimer, following the cross-linking of the individual complexes by using dithiobispionimidate with reducible disulfide bond. Then the dendriplex was PEGylated to improve the stability and finally conjugated with the luteinizing hormonereleasing hormone (LHRH) peptide via maleimide building block. These targeting dendriplexes preferentially knocked down B-cell lymphoma 2 genes in positive LHRH cells *in vitro*. For the investigation of *in vivo* gene efficiency of the siRNA carrier, the results showed a predominance of labeled siRNA and dendrimer in tumor tissue. The ability of functionalization of the periphery of the dendrimer with exquisite control allows its use as a vector for both DNA and siRNA transfections. Cationic dendrimers typically produce cytotoxic effect but also possess the ability to replace cations on the dendrimer by conjugating neutral or hydrophobic groups and then to append a targeting agent for an active targeting gene delivery. However, the gene-silencing activity *in vivo* still needs to be proved.¹⁰⁸

Promotion of the gene transfection is one of the key aspects in gene delivery. This can be accomplished by developing nontoxic gene deliveries that are controllably responsive. An innovative strategy based on photochemical internalization (PCI) gene delivery could provide time- and space-controlled endosomal escape of therapeutic molecules.¹⁰⁹ Ternary complexes with a size of around 100 nm were composed of cationic peptide (C(YGRKKRRQRRRG)₂) in the core that was enveloped in the second-generation aryl ether dendrimer (32 carboxyl groups at the periphery). The study of *in vitro* transfection denoted that this ternary system indicated the enhanced transgene express (>100-fold), when compared with the conventional reagents such as polyethyleneimine and lipofectamine. Fluorescence microscopic studies of rat conjunctival tissue revealed the positively induced gene delivery by PCI *in vivo*. This work demonstrated that the polyplex of polycations significantly affects both transfection efficiency and toxicity. Thus, PCI provides an

opportunity to develop an efficient light-inducible gene delivery system. Phototriggered gene transfection consisting of porphyrin and PAMAM dendrimer is also reported.¹¹⁰ In vitro results indicated that PAMAM dendrimers with porphyrin core were able to make complexes with green fluorescent protein plasmid DNA and that the internalization of dendrimers in HeLa was concentration dependent. In addition, the conjugation of porphyrin in the core of the dendrimer did not exhibit photocytoxicity at a concentration below 20 μ M. However, the expression of enhanced green fluorescent protein was enhanced in HeLa after PCI treatment.

VI. Dendrimers as Imaging Agents for Inspection

Polymer-based bioimaging probes for the diagnoses of different diseases were generated with the cooperation of polymer science and imaging science. The ultimate goal for *in vivo* bioimaging is to achieve highly sensitive and confident imaging systems for detecting and monitoring drug delivery. New probes with enhanced capabilities and performances should be developed based on nano-imaging technology. Key research of targeting *in vivo* bioimaging should address the design of nanostructures for extending the circulation time in the blood and preventing nanostructures from being recognized and cleared by macrophage before reaching the target cell. In addition, nanocarriers for imaging should selectively target diseased cells, tissues, and organs. Finally, new probes must exhibit compatibility with external activation of magnetic field, X-ray, or optics to trigger and enhance the monitoring ability.

A. Molecular Probes

Dendrimers are attractive molecules to be used as molecular probes due to their distinct morphology and unique properties. Large void volume and high density of functional groups in dendrimers make them very efficient to integrate molecular probes. Pt-coordinated complexes with peripheral functional groups were reported. The Pt complexes change to five-coordination Pt complexes via the electronic absorption of SO₂ adducts. The five-coordination Pt complexes could be easily recovered to the original state due to the steric repulsion between the coordination sites.¹¹¹ The reversible association of avidin on a biotin-functionalized dendrimer monolayer was reported, and its association behavior was monitored by cyclic voltammetry.¹¹²

Biosensors for DNA hybridization hold great promise for the rapid diagnosis of genetic diseases. Such sensors rely on the immobilization of single-stranded oligonucleotide probes that selectivity recognizes their complementary target tissue through hybridization. Most of the nucleotide-based

126

and DNA-based dendrimers have been synthesized for signal hybridization. A fluorescence oligonucleotide dendrimer on the signal amplification system was modified by using microarray technology in order to improve signal detection on DNA microarrays.¹¹³ The G4 dendrimer-conjugated 30 DNA single strands were immobilized onto a quartz crystal microbalance.¹¹⁴ Bioactivity of waterborne pathogen cryptosporidium parvum could be detected through the mass sensitive piezoelectric transducers. A large resonant frequency was detected, while target DNA formed three-dimensional surface hybridization. Immobilized dendrimers on the molecular probe were found to contribute to a higher sensitivity and wider linear range for biosensor, when compared with a non-dendrimer-immobilized probe. Finally, dendrimers must be water-soluble and be protected from interaction with biomolecules in the blood. Therefore, it could be applied in dendritic sensors for in vivo application. The Pd complexes of tetrabenzoporphyrins with amino acidbased dendritic wedge-modified chain end with PEG have been synthesized for *in vivo* oxygen imaging.^{115,116} These dendrimers have strong oxygendependent phosphorescence around 800 nm.

An amperometric enzyme electrode was developed for glucose sensor.¹¹⁷ Highly sensitive electrodes were fabricated by immobilizing glucose oxidase onto carbon and platinum electrodes and then by modifying with ferrocene colbaltoceium dendrimers. Results indicated that higher-generation dendrimers were more efficient as electron transfer mediators and can be applicable as sensors. In another study, the core of a PPI dendrimer functionalized with octamethylferrocenyl promotes the redox reaction of hydrogen peroxide.¹¹⁸ These dendrimer-based biosensors possess a good linear response of glucose detection, superior sensitivity, and data reproducibility as compared with other ferrocene-mediated glucose sensors.

Detection of human erythrocyte membrane acetylcholinesterase (AChE) was evaluated from the G4 PAMAM dendrimer-immobilized sensor.¹¹⁹ Low concentration of dendrimer at 25 μ M caused statistical enhancement in enzyme activity. Inhibition of AChE was observed at a concentration of dendrimer higher than 100 μ M. These inhibitions were found for both amine and hydroxyl-terminated PAMAM dendrimers. One explanation is attributed to the change of AChE activity caused by the alteration of protein by the addition of dendrimer. Another study aimed to clarify whether AChE inhibition was a result of direct action of the dendrimer on the enzyme or direct change on lipid bilayer.¹²⁰ It was demonstrated that anionic and cationic dendrimers change the AChE conformation and the strongest effect was observed by carboxyl-terminated G3.5 PAMAM dendrimers. Therefore, the changes in AChE conformation and catalytic activity depended on not only the concentration of the dendrimer but also its type.

The binding of PAMAM dendrimer to sodium hyaluronate (NaHA) has been investigated.¹²¹ Static light scattering denoted that when the dendrimer was bound, NaHA varied scarcely at low [NH₂]/[COO—] ratio, but the structure of NaHA changed to rodlike at high [NH₂]/[COO—] ratio. Electrostatic interaction between PAMAM dendrimers and NaHA is essential but not sufficient. The additional reason for effectively combining such a number of dendrimers on NaHA is the hydrogen bonding between dendrimer and NaHA.

B. Magnetic Resonance Imaging

Rapid diagnoses of genetic and pathogenetic diseases have been made possible by dendrimer-based biosensors. Complexes of PAMAM dendrimer loaded 1111n or 153Gd have been used as anchoring agents for specific and radiolabeled monoclonal antibodies. Gadolinium is a Food and Drug Administration (FDA)-approved contrast agent for magnetic resonance imaging (MRI) and it could provide great contrast between normal tissue and abnormal tissue in the body. Gadolinium is safer than iodine-type contrast used in computed tomography (CT) scans and also is rapidly cleared by the kidney. MRI is one of the prominent noninvasive diagnostic tools for disease detection and is based on subtle difference of environmentally sensitive proton nuclear magnetic resonance (¹H NMR) in the living system. The image of MRI depends on the inhomogeneous relaxation time of protons in different tissues.¹²²

The first imaging using the dendrimer-based MRI contrast agent for in vivo diagnosis was reported in 1994.¹²³ The dendrimer-based imaging agent exhibited blood-pooling properties and extraordinary relaxivity values when making chelate complex with gadolinium ion, Gd³⁺ (Gd(III)), in comparison with the commercially available small molecule agent.¹²⁴ Many early works of dendrimer MRI reagent focused on the PAMAM dendrimer.¹²⁵ G2 and G6 PAMAM dendrimers were conjugated with diethylene triamine petaacetic acid (DTPA), which is a commonly used gadolinium chelate agent, to create a chelate structure with 11 and 170 DTPA groups. Based on NMR studies, the chelates with G2 and G6 dendrimers increased longitudinal relaxivity (r1) to 21 and 34 mM⁻¹s⁻¹, respectively. These values were four- and sixfold higher ion relaxivities than that of free Gd(III) DTPA (5.4 mM⁻¹s⁻¹). The increase in r1 was attributed to the increased rotational correlation from chelating structure in dendrimer. There was significant enhancement in half-life from 24 min for Gd(III) DTPA to 40 and 200 min for G2 and G6 dendrimers, respectively. Generation dependency of dendrimer-based MRI reagents offered dramatic enhancement on MRI contrast properties, which is also called "dendritic effect." The limitation of relation to generation and r1 (an intrinsic property for evaluating the efficiency of MRI contrast agent) is up to G7 at a given field and temperature. Results indicated that the slow water exchange in highergeneration (G > 7) dendrimeric Gd(III) chelates displayed a limiting factor in r1.

The effects of scaffold rigidity on relaxivity enhancement have also been further investigated. Complex formation of chelating agent could increase relaxivity by reducing the internal motion of the dendrimer. A series of PPI Gd(III) DTPA structures with rigid linker groups was synthesized.¹²⁶ A G5 PPI dendrimer exhibited a relaxivity of 29 mM⁻¹s⁻¹. A comparable study was performed with a similar chelating dendrimer but modified with a flexible group.¹²⁷ The results indicated that the relaxivity was reduced to 29 mM⁻¹s ⁻¹ due to larger Gd(III) movement allowedby a flexible linker. Physical properties of dendrimer based on contrast agents not only affect the imaging application but also influence the agent-related toxicity. The prolonged blood-pooling retention time of a dendrimer as an MR agent limited its clinical usage due to the increasing time for release of toxic Gd(III) ion from the dendrimer chelating agent. Although high-generation dendrimer-Gd(III) chelating revealed higher relaxivity, it exhibited higher contrast image. In addition, the smaller generation of dendrimer conjugates showed more rapid excretion from the body than the higher generation did. Therefore, it has been proposed that the low generation of MRI dendrimer agents is most suitable for clinical use due to their therapeutic lower risk of toxicity and their prolonged retention, compared with commercial MRI agents. Gadomer-17, which is currently in phase II clinical trial, is a polylysine dendrimer with a trimesic acid core and only 24 groups at their periphery for gadolinium-chelating conjugation.¹²⁸ Gadomer-17 is suitable for blood-pooling imaging and similar to the well-known Gd(III) DTPA poly(lysine), but showed a superior elimination rate, which could be attributed to the globular character of dendrimer derivatives. In a related study, the internal rotational flexibility of Gd(III) chelate in Gadomer-17 and the slow water exchange rate, where both reduce the proton relaxivity, are currently identified as the limiting factors for MRI application.¹²⁹

C. X-ray Imaging and CT

X-ray CT is one of the medical diagnosis tools which could be applied to detect several diseases or organs, such as artioschlerotic vasculature, tumor, infarct, lung, and kidney. Similar to MRI reagents, high molecular weight contrast agents are better for quantitative detection of disease lesions. However, most of the commercial contrast agents are iodinated compounds. These small molecules could be quickly cleared and equilibrated between the intravascular and extracellular compartments in the body. Therefore, in order to achieve a clear contrast image, high concentration of contrast agents have to be injected into patients. This might give rise to other side effects because high dosages of iodine compounds could harm healthy cells or tissues. Consequently, to develop longer circulation time and higher contrast images at a low dosage becomes an important issue in the evolution of novel CT contrast agents. Dendrimers have attracted a lot of attention in association with X-ray contrast

agents, since the agents (bismuth, tin, and iodine) could form organometallic complexes with the dendrimer.^{130,131} Triiodobenzene derivative conjugated with the G4 dendrimer results in a water-soluble iodinated dendrimer particle for use as CT imaging agents.¹³² Superior spatial resolution can be reached due to the high iodine load within the dendrimer.

More recently, gold nanoparticles have been considered possible materials for a CT contrast agent, since gold nanoparticles can attenuate X-rays.^{133–137} It has been proved that contrast agents made from gold nanoparticles exhibited better contrast properties than commercially available iodine contrast agents did. Dendrimers could play a role in the control of the dispersion and the size of gold nanoparticles during the nanotemplating process. Various functional group-terminated dendrimers were evaluated in their applications for in vivo use. Gold nanoparticles as a CT contrast agent were loaded in a PEGylated dendrimer.¹³⁸ The contrast effect of this system was higher than that of a commercial iodated agent, which might be easily excreted from the body. The blood pool and the heart were enhanced in the CT image within 5 min after injection. Moreover, the biodistribution of gold nanoparticles could be monitored by CT and the further irradiation by light at the accumulation site induced the photothermal effect. Additionally, a new contrast agent was prepared by combining two contrast agent elements of gold and iodine within a PAMAM dendrimer nanodevice for CT imaging application.¹³⁹ Significant enhancing effects in X-ray attenuation intensity were obtained by combining multiple radio-dense elements in the dendrimer system.

A single photon emission computed tomography (SPECT) scan is another type of nuclear imaging test that reveals how the blood flows to tissues and organs. A SPECT scan integrates two technologies to view the body: (1) CT and (2) radioactive materials acting as a tracer. The tracer allows visualizing the flow of blood to tissue and organs. Technetium-99m (99mTc) is so far the most widely used radioactive tracer isotope in nuclear imaging. More than 80% of all usually used radiopharmaceuticals contain this short-lived metastable radionuclide. This is due to the highly characteristic physical properties of 99mTc, namely, short half-life (6.03 h) and γ photon emission of 140.5 keV, which is very important for both perspectives of effective imaging and patient safety. The 99mTc can be derived as a column eluted from a 99Mo/99mTc generator, which makes it readily available. Further, 99mTc possesses latent chemical properties, facilitating thereby the labeling of several types of kits for versatile diagnostic applications. In an approach to prepare well-defined poly(2,2-bis(hydroxymethyl)propanoic acid) dendron with reactive acid functionality at the core, it became possible to introduce a reactive ligand for 99mTc.¹⁴⁰ Å radioactive ligand in the dendron core was considered to minimize the interaction with the biological environment during the blood and tissue circulation. It was found that the radiochemical yield decreased with increasing generation

131

(G5 > G6 > G7), indicating the elimination of the reactivity by the isolating effect of the dendron. *In vivo* results showed that G5–G7 dendrimers were rapidly and efficiently removed from the bloodstream via the kidney and excreted through the bladder within 15 min post injection.

D. Optical Fluorescence Imaging

Biocompatible fluorescent molecules could perfectly work in tumor biosensing by using fluorescence detection techniques. This kind of approach provided an additional advantage when compared with nonbiocompatible techniques such as radiation or chemical analysis. In a related project, PAMAM dendrimer-based sensors were targeted to tumor cells to monitor the anticancer activity of therapeutics.¹⁴¹ Multifunctional folic acid-targeted PAMAM dendrimer delivery carriers were synthesized and covalently bound with apoptotic sensor, PhiPhiLux G1D2, in order to detect the extent of cell apoptosis. PhiPhiLux G1D2 is a caspase-specific Forster resonance energy transfer (FRET)-based agent that responds to the signal from an apoptosisinducing agent. A fivefold increase in intracellular fluorescence intensity was detected when dendrimers were internalized within the Jurkat cells in the first 30 min of incubation. This demonstrated the potential applicability of targeting the apoptosis-measuring dendrimer nanodevice, which could be simultaneously used for monitoring the apoptotic delivery of a drug *in vivo*.

Although conventional fluorescence optical images are limited due to the absorption and scattering of light in tissue, the elimination of light scattering and absorption could be accomplished by using an optical fiber that is placed into a doubtful tumor tissue to identify the region. Several biosensors based on one-photon fluorescence have been developed for the quantification of fluorescence materials in situ. Recently, two-photon system have been used for the simultaneous detection of fluorophores with a broad range of excitation wavelength with a spatial resolution of only a few micrometers. In addition, twophoton systems employ near-infrared light for excitation, which minimizes tissue damage, photobleaching, and intrinsic tissue fluorescence. Dendrimers were conjugated with 6-TAMRA, which is a two-photon excited fluorescencesensing agent, as a carrier to target xenograft tumors in mice. To quantify the concentration of a fluorescence agent in the tissue, a two-photon optical fiber could be inserted into tissue. This method has both advantages of being minimally invasive and being deeply sensitive in a live animal. The tumor fluorescence was observed in live mice at 0.5, 2, and 24 h with the help of a two-photon fluorescence probe. The results indicated that the dendrimer with folate targeting moiety showed selective accumulation in the tumor with maximum mean level at 673 ± 67 nM for 2 h after administration. The intensity of fluorescence for the non-targeting dendrimer as a control was at a level of 136 ± 28 nM for the same period and then decreased rapidly. Using the

two-photon excitation fiber-optic probe for detecting the dendrimer nanoparticle conjugated with 6-TAMRA, tumors containing as little as 0.3% fluorescent protein cells could be identified.¹⁴²

VII. Conclusions

The well-defined structure and multivalent periphery of dendrimers provide an excellent platform for attaching drugs, genes, targeting moieties, and imaging agents for biomedical and therapeutic applications. Although dendrimers have been expected to be a potential material in biomedical and pharmaceutical fields, the elimination of toxicity from dendrimers is an initial important issue. Nonselective interaction of cationic dendrimer systems caused membrane disruption and the erosion was followed by leakage of cytosolic enzymes and cell death. Reduction of the toxicity of dendrimers could be achieved by using the biocompatible anionic dendrimers or modifying the periphery of dendrimers.

The reactivity of dendrimers toward the terminal modification can achieve the high payload efficiency for bioactive agents. To avoid the drug release from physical loading, the drug must be conjugated on the periphery of dendrimers with the aim of preparing more stable drug delivery. Moreover, in order to apply to targeting therapy and diagnosis, anticancer bioactive and imaging agents are required for simple conjugation through chemical covalent bonding on the dendrimers. Dendrimers are also considered as an effective tool for various therapeutic applications such as neutron capture therapy, PDT, and gene therapy due to their unique structural architecture. In summary, dendrimers have achieved significant success in a variety of biomedical applications. Hopefully, this brief introduction of dendrimers toward biomedical applications clearly specifies perspectives for dendrimers in the emerging biomedical field.

References

- 1. Lundquist [], Toone E]. The cluster glycoside effect. Chem Rev 2002;102:555-78.
- Andre S, Ortega PJC, Perez MA, Roy R, Gabius HJ. Lactose containing starbust dendrimers: influence of dendrimer generation and binding site orientation of receptor (plant/animal lectins and immunoglobulins) on binding properties. *Glycobiology* 1999;9:1253–61.
- Chen HT, Neerman MF, Parrish AR, Simanek EE. Cytotoxicity, hemolysis, and acute in vivo toxicity of dendrimers based on melamine, candidate vehicles for drug delivery. J Am Chem Soc 2004;126:10044–8.
- Rittner K, Benavente A, Bompard-Sorlet A, Heitz F, Divita G, Brasseur R, et al. New basic membrane-destabilizing peptides for plasmid-based gene delivery in vitro and in vivo. *Mol Ther* 2002;5:104–14.

132

- Hong SP, Bielinska AU, Mecke A, Keszler B, Beals JL, Shi XY, et al. Interaction of poly (amidoamine) dendrimers with supported lipid bilayers and cells: hole formation and the relation to transport. *Bioconjug Chem* 2004;15:774–82.
- Stasko NA, Johnson CB, Schoenfisch MH, Johnson TA, Holmuhamedov EL. Cytotoxicity of polypropylenimine dendrimer conjugates on cultured endothelial cells. *Biomacromolecules* 2007;8:3853–9.
- Malik N, Wiwattanapatapee R, Klopsch R, Lorenz K, Frey H, Weener JW, et al. Dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of I-125-labelled poly(amidoamine) dendrimers in vivo. J Control Release 2000;65:133–48.
- Parimi S, Barnes TJ, Callen DF, Prestidge CA. Mechanistic insight into cell growth, internalization, and cytotoxicity of PAMAM dendrimers. *Biomacromolecules* 2010;11:382–9.
- 9. Jevprasesphant R, Penny J, Jalal R, Attwood D, McKeown NB, D'Emanuele A. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. *Int J Pharm* 2003;**252**:263–6.
- El-Sayed M, Ginski M, Rhodes C, Ghandehari H. Transepithelial transport of poly(amidoamine) dendrimers across Caco-2 cell monolayers. J Control Release 2002;81:355–65.
- Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. *Biomaterials* 2003;24:1121–31.
- Zinselmeyer BH, Mackay SP, Schatzlein AG, Uchegbu IF. The lower-generation polypropylenimine dendrimers are effective gene-transfer agents. *Pharm Res* 2002;19:960–7.
- Bourne N, Stanberry LR, Kern ER, Holan G, Matthews B, Bernstein DI. Dendrimers, a new class of candidate topical microbicides with activity against Herpes simplex virus infection. *Antimicrob Agents Chemother* 2000;44:2471–4.
- 14. Roberts JC, Bhalgat MK, Zera RT. Preliminary biological evaluation of poly(amidoamine) (PAMAM) starburst dendrimers. *J Biomed Mater Res* 1996;**30**:53–65.
- Padilla De Jesús OL, Ihre HR, Gagne L, Fréchet JMJ, Szoka Jr. FC. Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation. *Bioconjug Chem* 2002;13:453–61.
- Agrawal P, Gupta U, Jain NK. Glycoconjugated peptide dendrimers-based nanoparticulate system for the delivery of chloroquine phosphate. *Biomaterials* 2007;28:3349–59.
- Asthana A, Chauhan AS, Diwan P, Jain NK. Poly(amidoamine) (PAMAM) dendritic nanostructures for controlled site-specific delivery of acidic anti-inflammatory active ingredient. AAPS Pharm Sci Technol 2005;6:E536–42.
- Bhadra D, Bhadra S, Jain S, Jain NK. A PEGylated dendritic nanoparticulate carrier of fluorouracil. Int J Pharm 2003;257:111–24.
- Agashe HB, Dutta TD, Garg M, Jain NK. Investigations on the toxicological profile of functionalized fifth-generation poly (propylene imine) dendrimer. J Pharm Pharmacol 2006;58:1491–8.
- Carnahan MA, Grinstaff MW. Synthesis and characterization of polyether-ester dendrimers from glycerol and lactic acid. J Am Chem Soc 2001;123:2905–6.
- Dhanikula RS, Hildgen P. Conformation and distribution of groups on the surface of amphiphilic polyether-co-polyester dendrimers: effect of molecular architecture. J Colloid Interface Sci 2007;311:52–8.
- Hirayama Y, Nakamura T, Uehara S, Sakamoto Y, Yamaguchi K, Sei Y, et al. Synthesis and characterization of polyester dendrimers from acetoacetate and acrylate. Org Lett 2005;7:525–8.
- Agrawal A, Asthana A, Gupta U, Jain NK. Tumor and dendrimers: a review on drug delivery aspects. J Pharm Pharmacol 2008;60:671–88.

- Wegmann KW, Wagner CR, Whitham RH, Hinrichs DJ. Synthetic peptide dendrimers block the development and expression of experimental allergic encephalomyelitis. J Immunol 2008;181:3301–9.
- Cubillos C, De la Torre BG, Jakab A, Clementi G, Borras E, Barcena J, et al. Enhanced mucosal immunoglobulin A response and solid protection against foot-and-mouth disease virus challenge induced by a novel dendrimeric peptide. J Virol 2008;82:7223–30.
- Shao J, Tam JP. Unprotected peptides as building blocks for the synthesis of peptide dendrimers with oxime, hydrazone, and thiazolidine linkages. J Am Chem Soc 1995;117:3893–9.
- Konda SD, Aref M, Wang S, Brechbiel M, Wiener EC. Specific targeting of folate dendrimer MRI contrast agents to the high affinity folate receptor expressed in ovarian tumor xenografts. *Magn Reson Mater Phys Biol Med* 2001;12:104–13.
- Bhadra D, Yadav AK, Bhadra S, Jain NK. Glycodendrimeric nanoparticulate carriers of primaquine phosphate for liver targeting. Int J Pharm 2005;295:221–33.
- Gajbhiye V, Kumar PV, Tekade RK, Jain NK. PEGylated PPI dendritic architectures for sustained delivery of H2 receptor antagonist. *Eur J Med Chem* 2009;44:1155–66.
- Agrawal A, Gupta U, Asthana A, Jain NK. Dextran conjugated dendritic nanoconstructs as potential vectors for anti-cancer agent. *Biomaterials* 2009;30:3588–96.
- Kukowska-Latallo JF, Candido KA, Cao Z, Nigavekar SS, Majoros IJ, Thomas TP, et al. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Res* 2005;65:5317–24.
- Kolhatkar RB, Kitchens KM, Swaan PW, Ghandehari H. Surface acetylation of polyamidoamine (PAMAM) dendrimers decreases cytotoxicity while maintaining membrane permeability. *Bioconjug Chem* 2007;18:2054–60.
- Gajbhiye V, Kumar PV, Tekade RK, Jain NK. Pharmaceutical and biomedical potential of PEGylated dendrimers. *Curr Pharm Des* 2007;13:415–29.
- Dufes C, Uchegbu IF, Schatzlein AG. Dendrimers in gene delivery. Adv Drug Deliv Rev 2005;57:2177–202.
- Okuda T, Kawakami S, Akimoto N, Niidome T, Yamashita F, Hashida M. PEGylated lysine dendrimers for tumor-selective targeting after intravenous injection in tumor-bearing mice. J Control Release 2006;116:330–6.
- Morgan MT, Nakanishi Y, Kroll DJ, Griset AP, Carnahan MA, Wathier M, et al. Dendrimer encapsulated camptothecins: increased solubility, cellular uptake, and cellular retention afford enhanced anticancer activity in vitro. *Cancer Res* 2006;66:11913–21.
- Steffensen MB, Hollink E, Kuschel F, Bauer M, Simanek EE. Dendrimers based on [1,3,5]-triazines. J Polym Sci A Polym Chem 2006;44:3411–33.
- Chouai A, Simanek EE. Kilogram-scale synthesis of a second-generation dendrimer based on 1,3,5-triazine using green and industrially compatible methods with a single chromatographic step. J Org Chem 2008;73:2357–66.
- Twyman LJ, Beezer AE, Esfand R, Hardy MJ, Mitchell JC. The synthesis of water-soluble dendrimers, and their application as possible drug delivery systems. *Tetrahedron Lett* 1999;40:1743–6.
- Beezer AE, King ASH, Martin IK, Mitchel JC, Twyman LJ, Wain CF. Dendrimers as potential drug carriers; encapsulation of acidic hydrophobes within water soluble PAMAM derivatives. *Tetrahedron* 2003;59:3873–80.
- Kolhe P, Misra E, Kannan RM, Kannan S, Lieh-Lai M. Drug complexation, in vitro release and cellular entry of dendrimers and hyper-branched polymers. *Int J Pharm* 2003;259:143–60.
- Cheng Y, Xu T. Dendrimers as potential drug carriers. Part I. Solubilization of non-steroidal anti-inflammatory drugs in the presence of polyamidoamine dendrimers. *Eur J Med Chem* 2005;40:1188–92.

- Kannan S, Kolhe P, Raykova V, Glibatec M, Kannan RM, Lieh-Lai M, et al. Dynamics of cellular entry and drug delivery by dendritic polymers into human lung epithelial carcinoma cells. J Biomater Sci Polym Ed 2004;15:311–30.
- Cheng Y, Xu T, Fu R. Polyamidoamine dendrimers used as solubility enhancers of ketoprofen. Eur J Med Chem 2005;40:1390–3.
- Man N, Cheng Y, Xu T, Ding Y, Wang X, Li Z, et al. Dendrimers as potential drug carriers. Part II. Prolonged delivery of ketoprofen by in vitro and in vivo studies. *Eur J Med Chem* 2006;41:670–4.
- Recker J, Tomcik DJ, Parquette JR. Folding dendrons: the development of solvent-, temperature-, and generation-dependent chiral conformational order in intramolecularly hydrogen-bonded dendrons. J Am Chem Soc 2000;122:10298–307.
- Boas U, Karlsson AJ, de Waal BFM, Meijer EW. Synthesis and properties of new thiourea-functionalized poly(propylene imine) dendrimers and their role as hosts for urea functionalized guests. J Org Chem 2001;66:2136–45.
- Funayama K, Imae T, Aoi K, Tsutsumiuchi K, Okada M, Furusaka M, et al. Small-angle neutron scattering investigations of layer-block dendrimers in aqueous solutions. J Phys Chem B 2003;107:1532–9.
- Funayama K, Imae T, Seto H, Aoi K, Tsutsumiuchi K, Okada M, et al. Fast and slow dynamics of water-soluble dendrimers consisting of amido-amine repeating units by neutron spin echo. *J Phys Chem B* 2003;107:1353–9.
- Kannaiyan D, Imae T. pH dependent encapsulation of pyrene in PPI-core: PAMAM-shell dendrimers. Langmuir 2009;25:5282–5.
- Esfand R, Tomalia DA. Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. *Drug Discov Today* 2001;6:427–36.
- Ooya T, Lee J, Park K. Effects of ethylene glycol-based graft, star-shaped, and dendritic polymers on solubilization and controlled release of paclitaxel. J Control Release 2003;93:121–7.
- Leisner D, Imae T. Polyelectrolyte behavior of an interpolyelectrolyte complex formed in aqueous solution of a charged dendrimer and sodium poly(l-glutamate). J Phys Chem B 2003;107:13158–67.
- Tamano K, Imae T. Investigation of luminescent poly(propylene imine) dendrimer. J Nanosci Nanotechnol 2008;9:4329–34.
- Zheng Q, Pan CY. Preparation and characterization of dendrimer- star PNIPAAM using dithiobenzoate-terminated PPI dendrimer via RAFT polymerization. *Eur Polym J* 2006;42:807–14.
- Gurdag S, Khandare J, Stapels S, Matherly LH, Kannan RM. Activity of dendrimer-methotrexate conjugates on methotrexate-sensitive and -resistant cell lines. *Bioconjug Chem* 2006;17:275–83.
- 57. Lee CC, Gillies ER, Fox ME, Guillaudeu SJ, Fréchet JM, Dy EE, et al. A single dose of doxorubicin-functionalized bow-tie dendrimer cures mice bearing C-26 colon carcinomas. *Proc Natl Acad Sci USA* 2006;103:16649–54.
- Kolhe P, Khandare J, Pillai O, Kannan S, Lieh-Lai M, Kannan RM. Preparation, cellular transport, and activity of polyamidoamine based dendritic nanodevices with a high drug payload. *Biomaterials* 2006;27:660–9.
- Kono K, Kojima C, Hayashi N, Nishisaka E, Kiura K, Watarai S, et al. Preparation and cytotoxic activity of poly(ethylene glycol)- modified poly(amidoamine) dendrimers bearing adriamycin. *Biomaterials* 2008;29:1664–75.
- Najlah M, Freeman S, Attwood D, D'Emanuele A. Synthesis, characterization and stability of dendrimer prodrugs. *Int J Pharm* 2006;308:175–82.

- Najlah M, Freeman S, Attwood D, D'Emanuele A. In vitro evaluation of dendrimer prodrugs for oral drug delivery. Int J Pharm 2007;336:183–90.
- Kurtoglu YE, Mishra MK, Kannan S, Kannan RM. Drug release characteristics of PAMAM dendrimer–drug conjugates with different linkers. *Int J Pharm* 2010;384:189–94.
- 63. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov 2003;2:347-60.
- Hong S, Leroueil PR, Majoros IJ, Orr BG, Baker Jr. JR, Banaszak Holl MM. The binding avidity of a nanoparticle-based multivalent targeted drug delivery platform. *Chem Biol* 2007;14:107–15.
- 65. Chandrasekar D, Sistla R, Ahmad FJ, Khar RK, Diwan PV. The development of folate–PAMAM dendrimer conjugates for targeted delivery of anti-arthritic drugs and their pharmacokinetics and biodistribution in arthritic rats. *Biomaterials* 2007;28:504–12.
- Patri AK, Myc A, Beals J, Thomas TP, Bander NH, Baker Jr. JR. Synthesis and in vitro testing of J591 antibody–dendrimer conjugates for targeted prostate cancer therapy. *Bioconjug Chem* 2004;15:1174–81.
- Shukla R, Thomas TP, Peters JL, Desai AM, Kukowska-Latallo J, Patri AK, et al. HER2 specific tumor targeting with dendrimer conjugated anti-HER2 mAb. *Bioconjug Chem* 2006;17:1109–15.
- Wangler C, Moldenhauer G, Eisenhut M, Haberkorn U, Mier W. Antibody-dendrimer conjugates: the number, not the size of the dendrimers, determines the immunoreactivity. *Bioconjug Chem* 2008;19:813–20.
- Woller EK, Cloninger MJ. Mannose functionalization of a sixth generation dendrimer. Biomacromolecules 2001;2:1052–4.
- Roy R, Baek MG. Glycodendrimers: novel glycotope isosteres unmasking sugar coding. Case study with T-antigen markers from breast cancer MUC1 glycoprotein. J Biotechnol 2002;90:291–309.
- Lagnoux D, Darbre T, Schmitz ML, Reymond JL. Inhibition of mitosis by glycopeptide dendrimer conjugates of colchicines. *Chemistry* 2005;11:3941–50.
- Gabius HJ, Schroter C, Gabius S, Brinck U, Tietze LF. Binding of T-antigen-bearing neoglycoprotein and peanut agglutinin to cultured tumor cells and breast carcinomas. J Histochem Cytochem 1990;38:1625–31.
- Soloway AH, Tjarks W, Barnum BA, Rong FG, Barth RF, Codogni IM, et al. The chemistry of neutron capture therapy. *Chem Rev* 1998;98:1515–62.
- Hawthorne MF, Maderna A. Applications of radiolabeled boron clusters to the diagnosis and treatment of cancer. *Chem Rev* 1999;99:3421–34.
- Barth RF, Adams DM, Soloway AH, Alam F, Darby MW. Boronated starburst dendrimermonoclonal antibody immunoconjugates: evaluation as a potential delivery system for neutron capture therapy. *Bioconjug Chem* 1994;5:58–66.
- Capala J, Barth RF, Bendayan M, Lauzon M, Adams DM, Soloway AH, et al. Boronated epidermal growth factor as a potential targeting agent for boron neutron capture therapy of brain tumors. *Bioconjug Chem* 1996;7:7–15.
- Wu G, Barth RF, Yang W, Chatterjee M, Tjarks W, Ciesielski MJ, et al. Site-specific conjugation of boron-containing dendrimers to anti-EGF receptor monoclonal antibody cetuximab (IMC-C225) and its evaluation as a potential delivery agent for neutron capture therapy. *Bioconjug Chem* 2004;15:185–94.
- Barth RF, Wu G, Yang W, Binns PJ, Reley KJ, Patel H, et al. Neutron capture therapy of epidermal growth factor (+) gliomas using boronated cetuximab (IMC-C225) as a delivery agent. Appl Radiat Isot 2004;61:899–903.
- Ackroyd R, Kelty C, Brown N, Reed M. The history of photodetection and photodynamic therapy. *Photochem Photobiol* 2001;74:656–69.

- Najlah M, D'Emanuele A. Synthesis of dendrimers and drug-dendrimer conjugates for drug delivery. Curr Opin Drug Discov 2007;10:756–67.
- Battah S, Chee CE, Nakanishi H, Gerscher S, Macrobert AJ, Edwards C. Synthesis and biological studies of 5-aminolevulinic acid-containing dendrimers for photodynamic therapy. *Bioconjug Chem* 2001;12:980–8.
- Di Venosa GM, Casas AG, Battah S, Dobbin P, Fukuda H, Macrobert AJ, et al. Investigation of a novel dendritic derivative of 5- aminolaevulinic acid for photodynamic therapy. Int J Biochem Cell Biol 2006;38:82–91.
- Battah S, O'Neill S, Edwards C, Balaratnam S, Dobbin P, MacRobert AJ. Enhanced porphyrin accumulation using dendritic derivatives of 5-aminolaevulinic acid for photodynamic therapy: an in vitro study. Int J Biochem Cell Biol 2006;38:1382–92.
- Battah S, Balaratnam S, Casas A, O'Neill S, Edwards C, Batlle A, et al. Macromolecular delivery of 5-aminolaevulinic acid for photodynamic therapy using dendrimer conjugates. *Mol Cancer Ther* 2007;6:876–85.
- Gerhardt SA, Lewis JW, Kliger DS, Zhang JZ, Simonis U. Effect of micelles on oxygenquenching processes of triplet-state parasubstituted tetraphenylporphyrin photosensitizers. *J Phys Chem A* 2003;107:2763–7.
- Zhang GD, Harada A, Nishiyama N, Jiang DL, Koyama H, Aida T, et al. Polyion complex micelles entrapping cationic dendrimer porphyrin: effective photosensitizer for photodynamic therapy of cancer. J Control Release 2003;93:141–50.
- Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 1986;46:6387–92.
- Hirsch LR, Gobin AM, Lowery AR, Tam F, Drezek RA, Halas NJ, et al. Metal nanoshells. Ann Biomed Eng 2006;34:15–22.
- Shi X, Wang S, Meshinchi S, Van Antwerp ME, Bi X, Lee I, et al. Dendrimer-entrapped gold nanoparticles as a platform for cancer-cell targeting and imaging. *Small* 2007;3:1245–52.
- Haba Y, Kojima C, Harada A, Ura T, Horinaka H, Kono K. Preparation of poly(ethylene glycol)-modified poly(amido amine) dendrimers encapsulating gold nanoparticles and their heat-generating ability. *Langmuir* 2007;23:5243–6.
- Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001;411:494–8.
- Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998;391:806–11.
- Cristiano RJ, Xu B, Nguyen D, Schumacher G, Kataoka M, Spitz FR, et al. Viral and nonviral gene delivery vectors for cancer gene therapy. *Cancer Detect Prev* 1998;22:445–54.
- Ma H, Diamond SL. Nonviral gene therapy and its delivery systems. Curr Pharm Biotechnol 2001;2:1–17.
- Sonawane ND, Szoka Jr. FC, Verkman AS. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. J Biol Chem 2003;278:44826–31.
- Mitra A, Imae T. Nanogel formation consisting of dna and poly(amido amine) dendrimer studied by static light scattering and atomic force microscopy. *Biomacromolecules* 2004;5:69–73.
- Braun CS, Vetro JA, Tomalia DA, Koe GS, Koe JG, Middaugh CR. Structure/function relationships of polyamidoamine/DNA dendrimers as gene delivery vehicles. J Pharm Sci 2005;94:423–36.
- Kukowska-Latallo JF, Bielinska AU, Johnson J, Spindler R, Tomalia DA, Baker Jr. JR. Efficient transfer of genetic material into mammalian cells using Starburst polyamidoamine dendrimers. *Proc Natl Acad Sci USA* 1996;93:4897–902.

- 99. Wang Y, Kong W, Song Y, Duan Y, Wang L, Steinhoff G, et al. Polyamidoamine dendrimers with a modified pentaerythritol core having high efficiency and low cytotoxicity as gene carriers. *Biomacromolecules* 2009;10:617–22.
- Morgan DM, Larvin VL, Pearson JD. Biochemical characterisation of polycation-induced cytotoxicity to human vascular endothelial cells. *J Cell Sci* 1989;94:553–9.
- Arima H, Motoyama K. Recent findings concerning PAMAM dendrimer conjugates with cyclodextrins as carriers of DNA and RNA. Sensor 2009;29:6346–61.
- 102. Tsutsumi T, Hirayama F, Uekama K, Arima H. Evaluation of polyamidoamine dendrimer/ cyclodextrin conjugate (generation 3, G3) as a novel carrier for small interfering RNA (siRNA). J Control Release 2007;119:349–59.
- 103. Tsutsumi T, Hirayama F, Uekama K, Arima H. Potential use of polyamidoamine dendrimer/cyclodextrin conjugate (generation 3, G3) as a novel carrier for short hairpin RNA expressing plasmid DNA. J Pharm Sci 2008;97:3022–34.
- 104. Choi JS, Nam K, Park J-Y, Kim J-B, Lee J-K, Park J-S. Enhanced transfection efficiency of PAMAM dendrimer by surface modification with L-arginine. J Control Release 2004;99:445–56.
- Nam HY, Nam K, Hahn HJ, Kim BH, Lim HJ, Kim HJ, et al. Biodegradable PAMAM ester for enhanced transfection efficiency with low cytotoxicity. *Biomaterials* 2009;30:665–73.
- 106. Kim T-I, Bai CZ, Nam K, Park J-S. Comparison between arginine conjugated PAMAM dendrimers with structural diversity for gene delivery systems. J Control Release 2009;136:132–9.
- Kim KS, Lei Y, Stolz DB, Liu D. Bifunctional compounds for targeted hepatic gene delivery. Gene Ther 2007;14:704–8.
- Taratula O, Garbuzenko OB, Kirkpatrick P, Pandya I, Savla R, Pozharov VP, et al. Surfaceengineered targeted PPI dendrimer for efficient intracellular and intratumoral siRNA delivery. J Control Release 2009;140:284–93.
- Nishiyama N, Iriyama A, Jang WD, Miyata K, Itaka K, Inoue Y, et al. Light-induced gene transfer from packaged DNA enveloped in a dendrimeric photosensitizer. *Nat Mater* 2005;4:934–41.
- Shieh MJ, Peng CL, Lou PJ, Chiu CH, Tsai TY, Hsu CY, et al. Non-toxic phototriggered gene transfection by PAMAM-porphyrin conjugates. *J Control Release* 2008;**129**:200–6.
- 111. Albrecht M, Gossage RA, Lutz M, Spek AL, van Koten G. Diagnostic organometallic and metallodendritic materials for SO2 gas detection: reversible binding of sulfur dioxide to arylplatinum(II) complexes. *Chem Eur J* 2000;**6**:1431–45.
- 112. Daniel MC, Ruiz J, Nlate S, Blais JC, Astruc D. Nanoscopic assemblies between supramolecular redox active metallodendrons and gold nanoparticles: synthesis, characterization, and selective recognition of H2PO4-, HSO4-, and adenosine-5'-triphosphate (ATP2-) anions. J Am Chem Soc 2003;125:2617–28.
- Stears RL, Getts RC, Gullans SR. A novel, sensitive detection system for high-density microarrays using dendrimer technology. *Physiol Genomics* 2000;3:93–9.
- Wang J, Jiang M, Nilsen TW, Getts RC. Dendritic nucleic acid probes for DNA biosensors. J Am Chem Soc 1998;120:8281–2.
- Finikova O, Galkin A, Rozhkov V, Cordero M, Haegerhaell C, Vino-gradov S. Porphyrin and tetrabenzoporphyrin dendrimers: tunable membrane-impermeable fluorescent pH nanosensors. J Am Chem Soc 2003;25:4882–93.
- Rietveld IB, Kim E, Vinogradov SA. Dendrimers with tetrabenzoporphyrin cores: near infrared phosphors for in vivo oxygen imaging. *Tetrahedron Lett* 2003;59:3821–31.
- 117. Alonso B, Armada PG, Losada J, Cuadrado I, González B, Casado CM. Amperometric enzyme electrodes for aerobic and anaerobic glucose monitoring prepared by glucose oxidase

immobilized in mixed ferrocene-cobaltocenium dendrimers. *Biosens Bioelectron* 2004;**19**:1617–25.

- Armada MPG, Losada J, Zamora M, Alonso B, Cuadrado I, Casado CM. Electrocatalytical properties of polymethylferrocenyl den drimers and their applications in biosensing. *Bioelectrochemistry* 2006;69:65–73.
- 119. Klajnert B, Sadowska M, Bryszewska M. The effect of polyamidoamine dendrimers on human erythrocyte membrane acetylcholinesterase activity. *Bioelectrochemistry* 2004;**65**:23–6.
- 120. Shcharbin D, Jokiel M, Klajnert B, Bryszewska M. Effect of dendrimers on pure acetylcholinesterase activity and structure. *Bioelectrochemistry* 2006;**68**:56–9.
- 121. Imae T, Hirota T, Funayama K, Aoi K, Okada M. Binding of poly(amido amine) dendrimer to sodium hyaluronate in aqueous NaCl solution. J Colloid Interface Sci 2003;263:306–11.
- 122. Stiriba S, Frey H, Haag R. Dendritic polymers in biomedical applications: from potential to clinical use in diagnostics and therapy. *Angew Chem Int Ed* 2002;**41**:1329–34.
- 123. Wiener EC, Brechbiel MW, Brothers H, Magin RL, Gansow OA, Tomalia DA, et al. Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents. *Magn Reson Med* 1994;31:1–8.
- 124. Tomalia DA, Reyna LA, Svenson S. Dendrimers as multi-purpose nanodevices for oncology drug delivery and diagnostic imaging. *Biochem Soc Trans* 2007;35:61–7.
- 125. Wiener EC, Auteri FP, Chen JW, Brechbiel MW, Gansow OA, Schneider DS, et al. Molecular dynamics of ion-chelate complexes attached to dendrimers. J Am Chem Soc 1996;118:7774–82.
- 126. Kobayashi H, Kawamoto S, Jo S-K, Bryant Jr. HL, Brechbiel MW, Star RA. Macromolecular MRI contrast agents with small dendrimers: pharmacokinetic differences between sizes and cores. *Bioconjug Chem* 2003;14:388–94.
- 127. Langereis S, de Lussanet QG, van Genderen MHP, Backes WH, Meijer EW. Multivalent contrast agents based on gadolinium-diethylenetriaminepentaacetic acid-terminated poly (propylene imine) dendrimers for magnetic resonance imaging. *Macromolecules* 2004;**37**:3084–91.
- 128. Herborn CU, Barkhausen J, Paetsch I, Hunold P, Mahler M, Shamsi K, et al. Coronary arteries: contrast-enhanced MR imaging with SH L 643A—experience in 12 volunteers. *Radiology* 2003;**229**:217–23.
- 129. Nicolle GM, Toth E, Schmitt-Willich H, Raduchel B, Merbach AE. The impact of rigidity and water exchange on the relaxivity of a dendritic MRI contrast agent. *Chem Eur J* 2002;8:1040–8.
- Schumann H, Wassermann BC, Schutte S, Velder J, Velder J, Aksu Y. Synthesis and characterization of water-soluble tin-based metallodendrimers. *Organometallics* 2003;22:2034–41.
- Krause W, Hackmann-Schlichter N, Maier FK, Muller R. Dendrimers in diagnostics. Top Curr Chem 2000;210:261–308.
- 132. Fu Y, Nitecki DE, Maltby D, Simon GH, Berejnoi K, Raatschen HJ, et al. Dendritic iodinated contrast agents with PEG-cores for CT imaging: synthesis and preliminary characterization. *Bioconjug Chem* 2006;**17**:1043–56.
- Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. Br J Radiol 2006;79:248–53.
- 134. Kim D, Park S, Lee J, Jeong Y, Jon S. Antibiofouling polymer-coated gold nanoparticles as a contrast agent for in vivo x-ray computed tomography imaging. J Am Chem Soc 2007;129:7661–5.
- 135. Cai QY, Kim SH, Choi KS, Kim SY, Byun SJ, Kim KW, et al. Colloidal gold nanoparticles as a blood-pool contrast agent for X-ray computed tomography in mice. *Invest Radiol* 2007;42:797–806.

TSAI AND IMAE

- 136. Xu C, Tung GA, Sun S. Size and concentration effect of gold nanoparticles on X-ray attenuation as measured on computed tomography. *Chem Mater* 2008;20:4167–9.
- 137. Jackson PA, Rahman WN, Wong CJ, Ackerly T, Geso M. Potential dependent superiority of gold nanoparticles in comparison to iodinated contrast agents. *Eur J Radiol* 2010;**75**:104–9.
- Kojima C, Yasuhito U, Mikako O, Harada A, Yasuhiro M, Kono K. X-ray computed tomography contrast agents prepared by seeded growth of gold nanoparticles in PEGylated dendrimer. *Nanotechnology* 2010;21:1–6.
- 139. Guo R, Wang H, Peng C, Shen M, Zheng L, Zhang G, et al. Enhanced X-ray attenuation property of dendrimer-entrapped gold nanoparticles complexed with diatrizoic acid. J Mater Chem 2011;21:5120–7.
- 140. Parrott MC, Benhabbour SR, Saab C, Lemon JA, Parker S, Valliant JF, et al. Synthesis, radiolabeling, and bio-imaging of high-generation polyester dendrimers. J Am Chem Soc 2009;131:2906–16.
- 141. Myc A, Majoros IJ, Thomas TP, Baker Jr. JR. Dendrimer-based targeted delivery of an apoptotic sensor in cancer cells. *Biomacromolecules* 2007;8:13–8.
- 142. Thomas TP, Myaing MT, Ye JY, Candido K, Kotlyar A, Beals J, et al. Detection and analysis of tumor fluorescence using a two-photon optical fiber probe. *Biophys J* 2004;**86**:3959–65.

140