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PAMAM Dendrimer Based Targeted Nano-Carrier for Bio-Imaging and Therapeutic agents

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Abstract

In the last several decades, researchers have focused on developing suitable drug carriers to deliver pharmaceutical agents to treat cancer diseases. PAMAM dendrimers have been studied as potential delivery systems to targeting, imaging, and/or deliver therapeutic agents specifically to diseased tissues because of their unique properties, such as: multiple functionalities at the periphery or in the cavity, biocompatibility, tunable size, and monodispersity. Anti-cancer agents may be incorporated into the interior void space or conjugated to the surface of PAMAM to enhance the delivery of cytotoxic drugs. In addition, targeting ligands can also be attached to the dendrimer surface to allow active targeting and minimize harm to normal cells. In summary, this review highlights the contributions of PAMAM dendrimers to the field of nanotechnology with the intent to aid researchers in exploring dendrimers for targeted drug delivery, contrasting and bio-image agents.

Key words: Dendrimer, Drug delivery, Bioimaging agent, PAMAM, Targeting ligands, Therapeutic agents.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, affecting more than 10 million new patients every year. For example, 1,658,370 new cancer cases and 589,430 cancer deaths occurred in the United States in 2015 according to statistical data from the American Cancer Society in the National Cancer Institute (NCI) of the US.[1] The 5-year survival rate for all cancers diagnosed in 2004-2010 is 68%, up from 49% in 1975-1977. The improvement in survival reflects both earlier diagnosis and improvements in treatment.

Currently, there are several ways to treat cancers including surgery, radiation and chemotherapy.[2] However, more than 90% chemotherapeutic drugs have been approved by the Food and Drug Administration (FDA) for clinical use, their efficacy continues to be severely hampered due to dose-limiting toxicity and patient morbidity.[3] In addition, chemotherapeutic drugs often result in seriously side effects and inefficient delivery to tumor tissues due to poor solubility, nonspecific biodistribution and systemic toxicity, poor oral bioavailability, and low therapeutic index.[4] To improve the biodistribution of cancer drugs, nanoparticles have been designed with optimized size and surface characteristics. Forexample, nanoparticles with a hydrodynamic diameter of 5-100 nm have optimal pharmacokinetic properties for *in vivo* applications.[5] Nano-sized drug carriers are generally removed from blood circulation more slowly than their larger counterparts since, larger particles are quickly opsonized and removed from the bloodstream by macrophages of the reticuloendothelial system.[6]

Now a days, polymeric nanoparticles have been extensively studied for their chemical and physical properties and widely used in drug delivery systems.[7-10] These polymeric nanoparticles are prepared from a variety of biocompatible polymers and can be formulated to transport pharmaceutical agents in a controlled and targeted fashion through further surface modification

with specific ligands.[11] As previously reviewed, several polymeric nanocarriers can be prepared from both natural polymers, such as albumin, hyaluronic acid, and chitosan, and synthetic polymers, such as poly acrylamide (PAA), poly lactic acid (PLA), poly glycolic acid (PGA), poly(lactide-co-glycolide) (PLGA) and dendrimers.[12]

The purpose of this review is to provide readers an overview of well-defined PAMAM dendrimer nanomaterials which are available and may be of interest for cancer-related medical applications such as drug delivery and bio-imaging modalities. Therefore, the current review mainly focuses on the recent publications related to the development of PAMAM dendrimers for targeting and bio-imaging-guided drug delivery, as well as multifunctional dendrimer nano-complexes. A brief introduction is also given for the in vitro and in vivo diagnosis of cancer and with a brief concluding remarks as well as future prospects of the topic.

2. Dendrimer

Dendrimers are a family of 3D nano-sized macromolecules exhibiting highly branched architecture initially reported by Fritz Vogtle in 1978 and synthesized by Donald Tomalia and George R. Newkome in the 1980s.[13-15] The name "dendrimer" comes from two Greek words *dendron*, which means "tree branch like", and *meros,* which means "part of", and was first discovered by Tomalia et al.[13] Dendrimer is considered as the fourth new class of polymer structure, that demonstrates significant application in nanomedicine including drug delivery due to its unique platform for the construction of various multifunctional carriers.[14, 16-23] Poly (amidoamine) (PAMAM) dendrimers are a family of highly branched and monodispersed synthetic macromolecules with well-defined structure and composition.[24, 25] These polymers have internal cavities and peripheral functional groups that can be modified to better localize

drugs and modulate dendrimer-drug interactions.[25-27] PAMAM dendrimers differ from classical random coil molecules in that they possess perfect nano architectures comprising of three different parts which confer fascinating advantages.[16] These include: a) initiator core; b) building blocks with several layers of repeating units which grow radially, or folds called generations and nomenclature as G0, G0.5, G1, G2, G3 and so on; c) multiple peripheral functional groups, as shown in Figure 1.

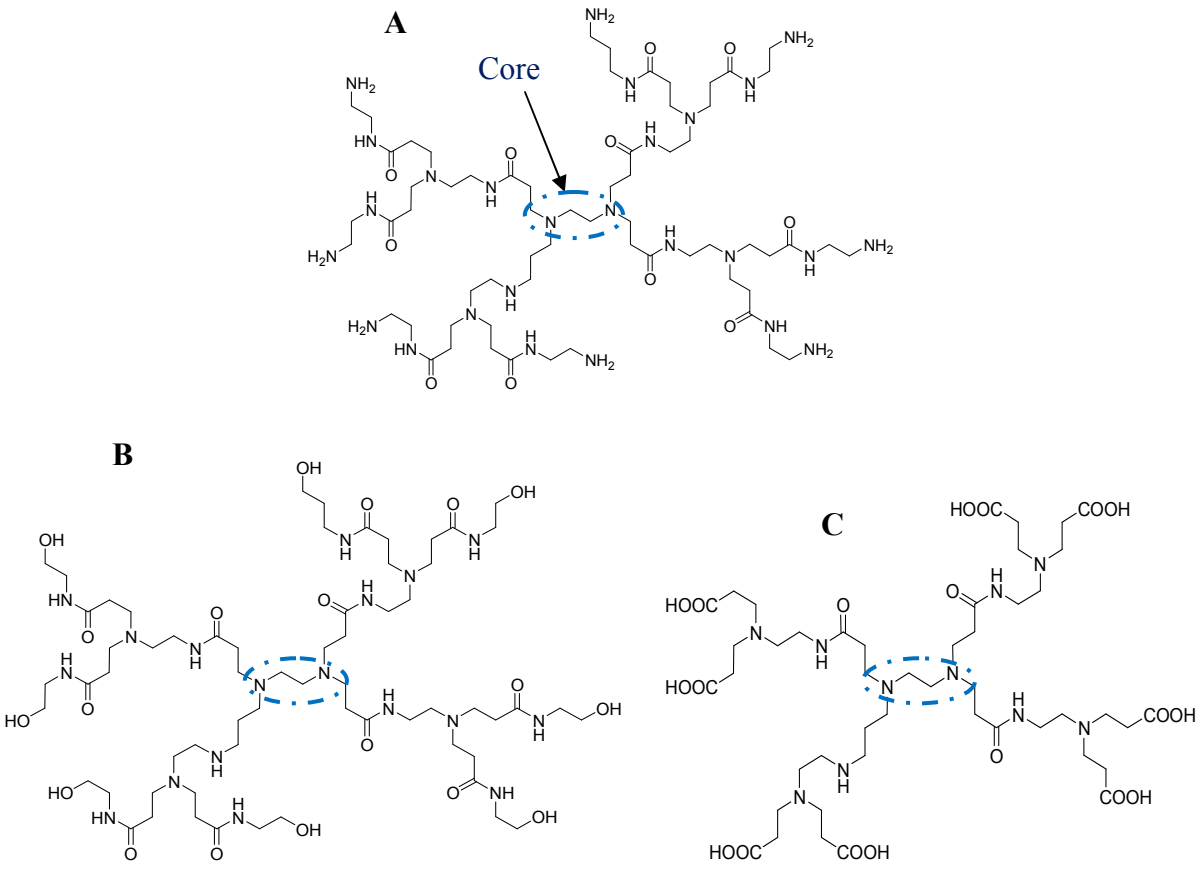

Figure 1: The dendrimer structure with an initiator core, interior layers of repeating units, and multiple peripheral functional groups (A) G1 with amine surface groups, (B) G1 with hydroxyl surface groups and (C) G0.5 with carboxyl surface groups.

2.1 Synthesis and properties of dendrimer

The first synthesis of a new class of topological macromolecules which referred to as "starburst polymers" is called dendrimer.[17] Dendrimer synthesis is accomplished by a variety of strategies and techniques. Among them, dendrimers are synthesized using a repetitive reaction sequence that flows a very highly monodisperse polymer chemistry and by virtue of their step-by-step controlled synthesis called molecular chemistry.[28, 29] Recent progresses in simplifying and optimizing the synthesis of dendrimers such as the 'lego' and 'click' approaches; provide a large variety of structures while at the same time reducing the cost of their production.[7, 30-32]

Generally, there are two approaches for dendrimer synthesis.[13, 28, 33] The first approach, called the divergent method, was developed by Tomalia, in which the growth of dendrimers start from the core and grows outward, building generation by generation. The second approach is the convergent method, first reported by Hawker and Fréchet, which proceeds from the dendron surface inward to a reactive focal point at the root.

It is generally accepted that the properties of dendrimers are essentially related to their terminal groups and that the internal structure is relatively insignificant.[25, 34] The starburst PAMAM dendrimers used today have an ethylenediamine core based on the reports of Tomalia *et al*.[16, 35] As compared to traditional linear polymers, dendrimers exhibit significantly improved physical and chemical properties. These properties include:

2.1.1. *Monodispersity*

Unlike linear polymers, dendrimers are intrinsically globular and can be constructed with well-defined monodispersed structure with highly modifiable surface groups. The

monodispersity of dendrimers has been confirmed by mass spectroscopy, gel permission chromatography (GPC) and transmission electron microscopy (TEM).[36]

2.1.2 Surface charge/Interior chemistry

The surface activities of PAMAM dendrimers in solution depend heavily on the location of the terminal groups, which may be either protonated or charged, and their distribution within the molecule. Surface chemistry of various generations of PAMAM dendrimers have been studied by different authors.[37, 38] The interactions of PAMAM dendrimers with plasma proteins have a significant impact on their *in vivo* transport and fate in circulation. The ability of polyamidoamine (PAMAM) dendrimers with different surface charges (positive, neutral and negative) to interact with a negatively charged protein (porcine pepsin) was studied.[39] According to the author, dendrimers with positive surface charge (G4 PAMAM-NH_2) and neutral surface charge (G4 PAMAM-OH) were able to inhibit the enzymatic activity of pepsin while dendrimer with negative surface charge (G3.5 PAMAM-COOH) was incapable of inhibiting enzyme activity of pepsin due to electrostatic repulsion between anionic dendrimer and the protein. Giri et al., reported that interactions of poly(amidoamine) dendrimers with human serum albumin (HSA).[40] They found that the HSA binding constants (Kb) of PAMAM dendrimers depend on size and terminal group chemistry. Another study reported that large cationic PAMAM dendrimers induced platelet aggregation via disruption of membrane integrity *in vitro* while anionic, neutral, and small cationic charged particles did not.[41] In addition to surface chemistry, the interior core of PAMAM is important for host-guest chemistry of dendrimer-drug complex in drug delivery systems. It is widely recognized that dendrimer interiors can be readily designed and reconfigured to provide a vast combinatorial array of special cavities, such as hydrophobic and hydrophilic domains, ligand domains, and acid-base complexation sites. Furthermore, higher

generation dendrimers have been shown to increase the drug load due to their large cargo space for guest molecules. An alternative approach of dendrimer self-assembly has also been applied to enhance the loading capacity of dendrimer-based carriers.[42]

2.1.3 Size and shape

The size of dendrimers varies depending on the generation, increasing systematically with the generation number. Low generation (G0-G3) PAMAM dendrimers with ethylenediamine core lack interior characteristics and are ellipsoidal in shape, whereas high generation (G4-G11) PAMAM dendrimers are spherical in size (1-15 nm) and have well-defined cavities.[13] Due to their dimensional length scaling, narrow size distribution, and other biomimetic properties, dendrimers are often referred to as "artificial proteins". Alternatively, generation 2, 3, 4, and 5 PAMAM dendrimers are similar in size with biological molecules such as DNA duplex width (2.4 nm), insulin (3 nm), cytochrome C (4 nm), and hemoglobin (5.5 nm) respectively. Moreover, generation 5 and 6 PAMAM dendrimers have diameters approximately equivalent to the thickness of the lipid bilayer membranes (~5.5 nm) of biological cells.[13, 43]

The dimensions of dendrimers and their response to solvent quality and composition can be determined from scattering data.[44] The size of dendrimers is largely influenced by the solvent quality.[44] Molecular dynamics reports demonstrated increasing internal segment density when the dendrimer-solvent interaction is less favorable, leading to a decrease in the average dimensions of the simulated structure.[44-46] At the molecular level, the size and shape of dendrimers can be measured using transmission electron microscopy (TEM), small-angle neutron scattering (SANS), and small-angle X-ray scattering (SAXS).[37, 47] In TEM, dendrimers appear as dark objects on a light background and are well dispersed from each other. Results

indicate that higher PAMAM generation dendrimers (G7-G10) are spherical in shape, with some molecules show "edges" or are slightly polyhedral.[36] The shape and size of G10 PAMAM dendrimers were studied by Cryo-TEM and shown to be more polyhedral and irregular. Structural properties of dendrimers such as inter-molecular structure, intra-molecular cavity, radius-of-gyration (RG), effective charge number of a single dendrimer molecule, and water penetration into the interior of the dendrimers have been explored by SANS.[48]

2.1.4 Biocompatibility

The nanosized particles like dendrimers have the ability to interact with nanometric cellular components such as cell membrane and cell organelles,[49] as a result its biocompatabilities were studied. Cationic dendrimers are generally more haemolytic and cytotoxic even at low concentrations than their anionic counterparts.[50-52] PAMAM dendrimers have recently been investigated as efficient and biocompatible oil dispersants [53]; however, their high cationic charge density was shown to be cytotoxic. Highly cationic PAMAM dendrimers are highly toxic to amoebas at high concentrations.[54] Similarly, other studies have shown that highly cationic PAMAM resulted in significant charge-induced toxicity [55, 56] and rapid blood clotting *in vivo*.[57] In addition, the influence of PAMAM dendrimer surface chemistry on Caco-2 cells was studied and results show that anionic PAMAM dendrimers (G2.5, G3.5) exhibited no measurable cytotoxicity up to 1 mM concentration. In contrast, cationic G2 PAMAM dendrimers were toxic toward Caco-2 cells at concentrations above 700 μM. On the other hand, G3 and G4 were significantly cytotoxic at all concentrations examined.[58] N. Malik et al., were studies the effect of PAMAM dendrimer generation and surface functionality on biological properties in vitro.[59] According to the report, dendrimers bearing -NH termini displayed concentration and generation-dependent haemolysis, and changes in red blood cell morphology were observed after

1 h even at low concentrations (10 mg/ml). Those dendrimers with carboxylate (COONa) terminal groups were neither haemolytic nor cytotoxic towards a panel of cell lines in vitro. This has been attributed to the electrostatic interaction between highly cationic PAMAM and negatively charged cell membrane which triggers toxicity by pore formation or nano-hole formation, membrane thinning, and erosion. Therefore, it is essential to mitigate cationic charge-induced toxicity through surface group modification such as the acetylation of terminal amines.[60] Cytotoxicity was reduced by more than 10-folds as acetylation degree increased and the permeability across cell monolayer was altered. Similary, Roberts et al., study shows that the toxicity of the PAMAMs is dose and generation dependent.[38] Generatio 5 or smaller PAMAM dendrimers do not appear to present any problem *in vivo* from a toxicity point of view. These results suggest that the larger generations of PAMAM dendrimers may not be a good choice for biological uses, while the smaller generations appear to have little or no deleterious effect at levels to be found in biological applications. Generally, the cytotoxicity of PAMAM dendrimers can be ranked in the order of hydroxyl-terminated < carboxyl-terminated < amine terminated systems. Carboxyl-terminated PAMAM of generations 3.5 and 4.5 (G3.5-COOH and G4.5-COOH) were toxic to cells only at high concentrations of 10.0 mM while amine-terminated PAMAM of generations 3.0 and 4.0 (G3.0-NH_2 and G4.0-NH_2) were toxic at 1.0 mM.[61] Since anionic PAMAM dendrimers are better tolerated, they can be used at higher doses than cationic PAMAM dendrimers. In the range of dendrimers evaluated from generations 0.0 to 4.0 with varying surface functional groups, there exists a workable non-toxic window for PAMAM dendrimers for use as carriers for oral drug delivery.[62]

2.3 Dendrimers as drug carrier

Dendrimers are attractive as drug carriers due to their highly branched 3D structure which can be used to carry a variety of cargos including therapeutic drugs, imaging agents, and nucleic acid materials.[19, 63] Dendrimers have been used in cancer-related studies as delivery systems for anticancer drugs and agents for boron neutron capture therapy and photodynamic therapy.[19, 24] The different types of interactions of dendrimers with drug molecules have been previously reviewed.[21] There are two major approaches for drug loading: (a) Attaching the drug covalently or by electrostatic interaction at the periphery of the dendrimer and (b) incorporating the drug in the interior of the dendrimer or by physical adsorption. The latter is more desirable because drug release can be controlled by diffusion whereas in the former case, suitable mechanisms are required to detach the drug.

2.3.1 Dendrimer-drug conjugates

Conventionally, drugs are attached directly via linkers or spacers to dendrimer terminal groups and, in some instances, in combination with targeting moieties. Drugs are often linked to dendrimers via hydrolysable or enzymatically cleavable bonds such as esters, amides, carbamates, and hydrazones.[64] Amide linkages can be broken down in the plasma as well as in the lysosomal compartment by peptidases or cathepsins.[65] On the other hand, ester and amide bonds are cleavable under general hydrolytic conditions. This allows better control over drug release compared to physical adsorption between dendrimer/drug complexes. In one study, the drug acetyl cysteine (NAC) was linked to PAMAM dendrimers possessing carboxylic and amine terminal groups via cleavable disulfide linkages using glutathione and *N*-succinimidyl 3-(2-pyridyldithio)-propionate, respectively. Results revealed that NAC was released in its active

form from NAC-PAMAM dendrimer conjugate via disulfide exchange reaction with indigenous intracellular glutathione. Clinical trial results showed that the system was more effective than the drug alone in treatment.[66, 67]

2.3.2 Dendrimer-drug encapsulation

It has been shown that dendrimers with a hydrophobic interior and hydrophilic chain ends are able to solubilize hydrophobic drugs in aqueous solutions by host guest interactions inside their empty cavities (void spaces).[68, 69] Also, small guest molecules can be physically entrapped in the interior of dendrimers with rigid shells, as reported previously.[70] Other studies indicate that dendrimers can act as unimolecular nanocapsules for small guest molecules.[71] A recent review reported that amine-terminated PAMAM dendrimers are able to solubilize different families of hydrophobic drugs.[72] The encapsulation of drug molecules or nanoparticles by dendrimers can be characterized by ^{1}H NMR, Fluorescence, TEM, UV-Visible and Fourier transform infrared spectroscopy.[26, 73] In our previous report, the encapsulation efficiency of doxorubicin (DOX) into the interior of G4.5 PAMAM dendrimer was studied by fluorescence emission spectroscopy.[8] In addition, the loading contents were studied by UV-visible and ^{1}NMR spectroscopy. The conjugation or encapsulation of drugs with PAMAM dendrimers provide increased solubility, biocompatibility, and desirable pharmacokinetic profile of the drug, resulting in greater efficacy in clinical applications. In addition to the PAMAM dendrimers alone, lipid-PAMAM dendrimers hybrid (LDH) nanosystem shows a synergic effect of drugs encapsulation efficiency. According to the recent report, the potency of paclitaxel could be significantly improved by 37-fold when presented in the LDH nanosystem as compared to free drug, whereby paclitaxel and PAMAM G4.0 acted synergistically in killing the ovarian cancer cells.[74]

2.4 Dendrimers for targeted drug delivery

Nanoparticles used for drug delivery are typically sized 5-380 nm and can be made from various materials.[75] These nano sized drug carriers are generally removed from the blood circulation more slowly than their larger counterparts.[6] However, nanoparticles smaller than 5 nm in hydrodynamic diameter are rapidly eliminated via the urinary system.[76] Nanoparticles based drug delivery systems are of great importance because they not only effectively improve pharmacokinetics and biodistribution, but also provide controlled release kinetics to the target site. Among those polymeric materials, the structural advantage of dendrimers allows them to play important role in the field of nanotechnology. The majority of preveous reviews also highlights about dendrimer's nanostructures represent as useful nano-carriers for medicine, and often referred to as the "polymers of the 21st century".[77-80] In addition to these, the present review highlights that the introduction of targeting ligands onto the 3D-dendrimer surfaces used for disease targeted carrier or "targeted delivery" were depicted.

Targeting of drugs in a cell, tissue, or disease-specific manner represents potentially powerful technology that can be used broadly in medicine, especially cancer.[81] Although cancer cells are inherently more vulnerable to chemotherapy than the majority of normal cells, anti-cancer drugs which are non-selective can cause injury to normal tissues. Targeted drug delivery can enhance the chemotherapeutic effect and prevent damage to normal tissues.

There are two types of nanocarrier targeting mechanisms that can be used to delivery drugs to the diseased site: passive targeting and active targeting as shown in Figure 2. Passive targeting implies that nanoparticles passively extravasate through leaky vasculature into the tumor tissue via enhanced permeability and retention (EPR) effect.[82-84] The EPR effect is a unique phenomenon that is dependent on the pathophysiological differences between tumor

microenvironments and normal tissues and serves as the most crucial strategy in improving the delivery of therapeutic agents to tumors for anticancer drug development.[85] This phenomenon takes advantage of both tissue physiological properties as well as nanoparticle physicochemical properties. Tumors typically have a leaky vasculature and impaired lymphatic drainage, which results in 10-30 times higher drug concentration in tumors than in blood. This is due to a homing effect to the diseased site that is driven solely by particles' nano dimensions through the EPR effect rather than any specific recognition. On the other hand, active targeting involves drug delivery to a specific site based on molecular recognition. On approach of the latter is to couple ligands which can interact with receptors on the target cell to nanoparticles and gives more enphasis in the review. Covalent conjugation of specific targeting moieties such as sugar,[86] folic acid, [19, 87-89] monoclonal antibodies,[90, 91] and peptides [92-94] to carriers confer receptor-mediated active targeting ability toward tumor tissues.

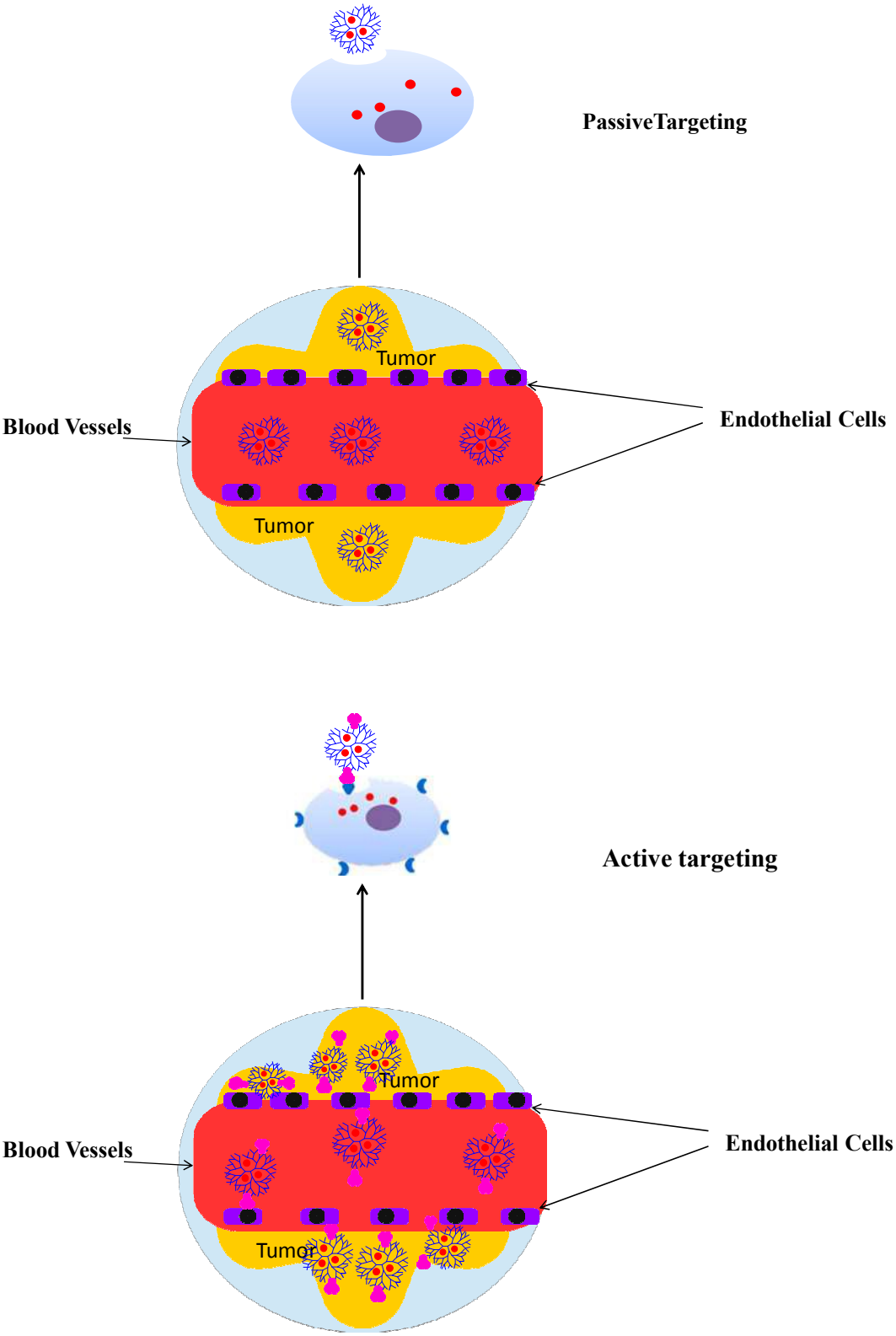

Figure 2: General scheme of passive and active targeting approaches of nanoparticles uptake by cancer cells. Passive tumor targeting is achieved by extravasations through leaky vasculature via enhanced permeability and retention (EPR) effect. Active tumor targeting can be achieved by functionalization of nanoparticles with targeting ligands that promote cell-specific recognition and binding.

2.4.1 Peptide targeted drug delivery

The Arg-Gly-Asp peptide is a promising ligand molecule for targeting the αvβ3 integrin which is over expressed in angiogenic sites and tumors.[95, 96] The high affinity interaction between RGD peptide and cancer-related integrin has been exploited for various targeted drug delivery and gene delivery applications. Dendrimers coupled to multiple *αvβ3* selective ligands (RGD-4C) for targeting to tumor-associated capillary bed have been shown to release cytotoxic agents and effectively killed newly formed vessels.[92, 97] PAMAM dendrimer-RGD conjugates have also been found to mediate cellular binding and adhesion. Furthermore, RGD-conjugated dendrimers are generally considered to be nontoxic to normal cells. These conjugates also possess the ability to deliver imaging agents to target carcinoma cells through cellular binding and adhesion.[97]

2.4.2 Monoclonal antibody-targeted delivery system

Monoclonal antibodies generated against specific antigens, when conjugated to dendrimers, can selectively deliver drugs to cancer cells while minimizing damage to normal cells shouldn't expression the specific ligand. Prostate specific membrane antigen (PSMA) J591 antibody was conjugated to G5 PAMAM and the in vitro studies of these conjugates show that they

specifically bind to cells expressing PSMA receptor.[98] In a separate study, the binding and internalization of interleukin-6 (IL-6) monoclonal anti-body conjugated dendrimer by HER2-expression cells was evaluated by flow cytometry and confocal microscopy.[91] Results from internalization and competitive experiments with free antibody suggest the rapid and efficient cellular internalization of the G4.5 PAMAM/IL-6 conjugate.[99] IL-6 is an important multifunctional cytokine that plays a crucial role in angiogenesis. Due to the rapid neovascularization of tumors, IL-6 is overexpressed in cancer cells. In this study, the high affinity of IL-6 for human epidermal growth factor receptor (HEGFR) results in the significant internalization of IL-6 modified complexes into HeLa cells through the receptor mediated endocytosis pathway.[8] Furthermore, drug liberation into the nucleus was also significantly higher for IL-6-conjugated complexes than native PAMAM due to the higher multivalency of IL-6 which strengthened ligand-receptor binding and increased targeting.[8, 20] Dendrimers have also been conjugated to anti-EGFR antibodies such as Cetuximab for enhanced selectivity and cellular uptake of antitumor drugs for the treatment of brain tumors.[100]

2.4.3 FA-targeted delivery system:

Folate (FA) is known to target FA receptors (FAR) that are over expressed in several human carcinomas and is one of the most studied targeting ligands toward cancer cells. The folate receptor is a tumor marker that binds vitamin folate and folate-drug conjugates with high affinity and carries these bounded molecules into the cell via receptor mediated endocytosis.[101, 102] A recent report demonstrated the loading of DOX into the interior of FA-conjugated G5 polypropylene imine dendrimers.[103] Another study revealed that a folic acid-containing DOX-loaded G5 dendrimer, where doxorubicin was caged, effectively targeted to tumor and the drug was released by a photocleavable mechanism.[104] Wang, Y., et al showed that G5 PAMAM

dendrimers with covalently attached FA enabled the intracellular delivery of the anticancer drug 2-methoxyestradiol to FAR-overexpressing cells, resulting in cancer cell death.[105]

2.4.4 Glycoprotein targeted drug delivery

The presence of lectin receptors on different cell surfaces allow glycosylated carriers to be used for targeted drug delivery. N-acetylgalactosamine (NAcGal) sugar molecules have been conjugated to the primary surface groups of G5-NH_2 terminated PAMAM dendrimers via peptide and thiourea linkages to provide G5-NAcGal conjugates with various sugar densities. These G5-NAcGal conjugates selectively bound asialoglycoprotein receptors (ASGPR), which are highly expressed on the surface of hepatic cancer cells, and triggered receptor-mediated endocytosis into hepatic cancer cells.[106] In a separate study, Huang, J., et al. reported the modification of a block copolymer composed for G2.5 PAMAM dendrimer and polyethylene glycol (PEG) with galactose (Gal) to allow receptor-mediated liver targeting for potential drug delivery. The release of DOX from the prodrug Gal-PEG-G2.5-DOX was accelerated when the pH was decreased from 8.0 to 5.6 and the cytotoxicity against BEL-7402 (Human hepatoma cell line) was lower than that of free DOX due to gradual release of the drug.[107]

2.4.5 Biotinylated targeted drug delivery

Biotin is an essential micronutrient that is essential for normal cellular functions such as fatty acid biosynthesis, gluconeogenesis, growth, and catabolism. More importantly, the level of biotin is relatively high in rapidly proliferating cells such as cancer cells; thus, specific interaction between biotin and its receptors may be exploited for targeted drug delivery.[108] One study reported the conjugation of partially acetylated G5 PAMAM with biotin to give a nanodevice for *in vitro* cancer targeting.[109] A separate study reported the preparation of biotinylated PAMAM

using sulfo-NHS-LC-biotin which was internalized by receptor-mediated endocytosis and charge-mediated adsorptive endocytosis. The interaction of biotinylated G4 PAMAM with the blood brain barrier (BBB) *in vitro* was evaluated using Langmuir Blodgett monolayer technique, atomic force microscopy (AFM), and lactate dehydrogenase.[110] Uptake studies revealed that these biotinylated dendrimers exhibited both charge mediated and sodium-dependent multivitamin transporter (SMVT) mediated uptake.[111] SMVT has been suggested to be responsible for biotin uptake and is expressed in other cells such as human keratinocytes, peripheral blood mononuclear, intestinal, liver, and renal epithelial cells.[112-114] Thus, biotinylation is an attractive strategy for targeting chemotherapeutic agents to cancer cells.

2.5 Dendrimers for Biomedical imaging

Nanoscopic molecular targeting and diagnostic imaging are emerging as the next generation of multifunctional nanomedicine and aim to improve the therapeutic outcomes of drug therapy.[115] Therefore, dendrimers are versatile tools for biomedical imaging and have been used for numerous modalities including magnetic resonance imaging (MRI), X-ray computed tomography (CT), optical imaging and nuclear medicine (neutron capture therapy for cancers). The unique branching architecture and high number of functional groups present on the surface allow the chelation or encapsulation of various metal ions for imaging (paramagnetic or radio opaque) and therapy (radioactive particle emitters).

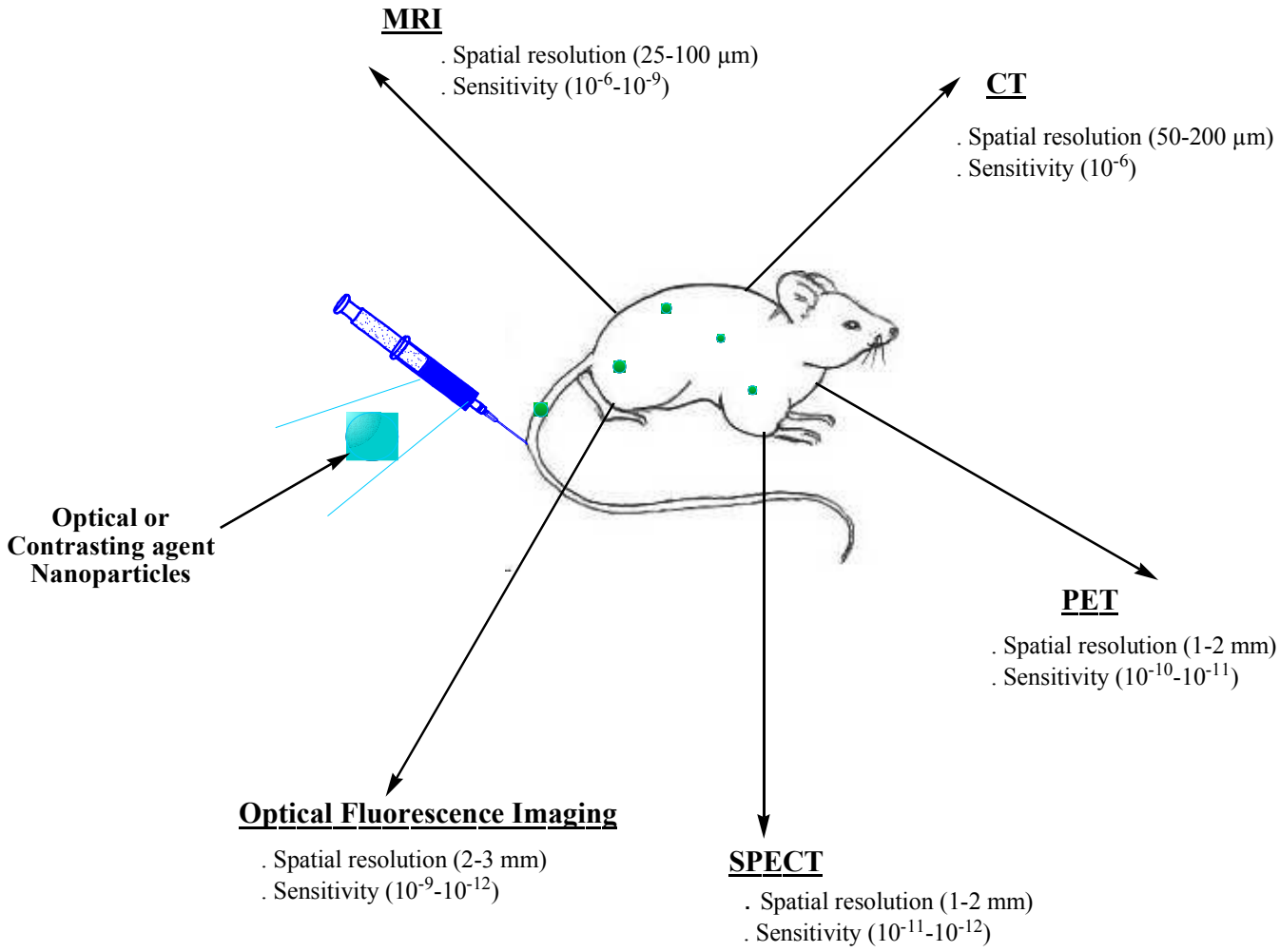

Figure 3: Several biomedical imaging modalities and potential applications of nanoparticles

2.5.1 Dendrimers for optical imaging.

The covalent attachment of fluorescent probes onto dendrimers or their cargos enables visualization under a fluorescent microscope. However, modification with organic fluorophores may result in decreased biocompatibility and changes to the size and mobility of dendrimers. A recent study has described the inherent blue photoluminescence originating from G4 PAMAM

dendrimers found that the emission intensity could be dramatically enhanced by oxidative treatments.[99, 116] Several studies are dedicated to exploring the fluorescent properties of dendrimers. Larson and Tucker *et al.*[117], were reports weak fluorescence from COOH terminated G2.5-G7.5 PAMAM dendrimers due to n-π* transition of the amido group. Similarly, Lee *et al*.[118], were observed strong photoluminescence in oxidized OH-terminated G0, G2, and G4 PAMAM dendrimers which suggests that the terminal –OH rather than the dendrimer backbone is responsible for the formation of luminescent. In addition, Wang et al[119] reported that strong fluorescence of -OH, -COOH, and NH_2-terminated G4, G4.5 and G4 PAMAM dendrimer, respectively in aqueous solution and the intensity was dependent on concentration and pH. Although fluorescent properties of dendrimers are becoming better understood, the effects of conjugation to macromolecules on fluorescent properties remain largely unexplored. Recently, we studied the cellular internalization and binding of G4.5-IL6 bioimaging probe prepared by modifying carboxyl terminated G4.5 PAMAM dendrimers with IL-6 via NHS/EDC coupling chemistry.[99] we observed that IL6 conjugation enhance the flourscence properties of G4.5 PAMAM dendrimer, which is due to the decrease in the pH of G4.5-IL6 complex.

2.5.2 Dendrimers for magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is a non-invasive, real-time method used to obtain three-dimensional tomographic information on anatomical details with high special resolution and soft tissue contrast. MRI contrast agents contain paramagnetic or super paramagnetic metal ions that affect the MRI signal property of surrounding tissues. Nanoparticles with magnetic imaging properties, such as those consisting of gadolinium (III) ion (Gd^{3+}), are used as paramagnetic MRI contrast agents. These contrast agents are capable of reducing the longitudinal relaxation time

(T_1) of the surrounding water protons. Clinically, MRI can be improved by using T1 positive and T2 negative MRI contrast agents.

Paramagnetic metal chelates such as Gd (III)-DOTA, Gd (III)-DTPA, and their derivatives increase the relaxation rate (r1) of surrounding water protons and are used as positive contrast agents for MRI to increase the signal intensity in T1-weighted MRI. Based on this understanding, the incorporation of Gd3+ ions into nanoscale carriers thereby enhances the relaxivity (r_1) of paramagnetic contrasting agents. Several nanoparticles and macromolecules, including polymers, proteins, dendrimers, micelles, and vesicles, have already been explored as platforms for Gd-labeling agents.[120] Since macromolecular contrast agents (>20000 Da) permit longer imaging time and possess higher relaxation rate due to their longer rotational correlation time, dendrimers are particularly well-suited.[121, 122] Furthermore, the addition of targeting ligand potentially increases the longitudinal relaxivity of intravenously injected contrast media.[123]

According to a previous study, dendrimer based macromolecular MRI contrasting agents of different sizes and properties can be prepared by simple chemistry and provide sufficient contrast enhancement for multiple applications.[124] Dendrimers can be used to either encapsulate or directly conjugate paramagnetic materials. The first new class of dendrimer based metal chelating MRI contrast agent with large proton relaxation enhancement and high molecular relaxivities was introduced by Wiener, E., *et al*.[125] One study revealed that a MRI contrast agent consisted of G4.0 PAMAM with folic acid as targeting moiety efficiently increased the longitudinal relaxation rate at T1 by over 100% in cells expressing the folate receptor, compared to untreated cells.[126] In a recent communication, gadolinium (Gd) labeled nanoclusters functionalized with folic acid ligands was reported as a tumor-targeted T1 MRI contrast agent *in vivo*.[127] According to the report, the folate receptor targeted Gd labeled G5 PAMAM dendrimer

nanoclusters increased the payload of gadolinium and enhanced longitudinal relaxivities (r1) per Gd. In addition to gadolinium chelates, gadolinium oxide (Gd_2O_3) nanoparticles have been exploited for their diagnostic and therapeutic properties.[128-133] According to Ahmad et al,[134] gadolinium oxide nanoparticles with dual imaging capacity can be used for MRI and CT. This dual functionality arises from the high spin magnetic moment of Gd^{3+} ($^8S_{7/2}$) as a result of its seven unpaired 4f-electrons.[134, 135]

Currently, most T1 MRI contrast probes used are based on gadolinium (Gd^{3+}) chelates. However, their usage has recently been associated with nephrogenic system fibrosis (NSF) [136, 137] which is fibrosis of the skin and internal organs, in patients who have renal insufficiency.[138] Recently, manganese (Mn)-based nanoparticles have emerged as a new class of probes for MRI due to their impressive contrast ability and safety.[139] These probes have been shown in a recent review to exhibit prominent contrasting effects, revealing detailed physiological and biological information not previously possible with conventional Gd(III)-based T1 contrast agents or super paramagnetic iron oxide (SPIO)-based T2 contrast agents.[120] Alternatively, the immunotoxicity of Mn based nanoparticles have been assessed pre-clinically and according to the report, Mn-based nanoprobes are safe and biocompatible both *in vitro* and *in vivo*.[140, 141] Although no work has focused on Mn^{2+} containing T1 weight paramagnetic contrasting agents, a cancer cell-targeting nanocarrier system was developed recently and used multifunctional G3 PAMAM dendrimers to produce folic acid functionalized manganese ferrite nanoparticles as T1 and T2 contrast agents.[142]

2.5.3 X-ray computed tomography (CT)

Although CT enables 3D anatomic imaging at high spatial resolution, the delivery of an X-ray contrast agent is required in order to distinguish tissues with similar or low X-ray attenuation. It allows excellent soft tissue contrast imaging to obtain anatomical and functional information. CT possesses higher spatial resolution and lower sensitivity in comparison to other clinical imaging modalities such as positron emission tomography (PET) and MRI. Metallic nanoparticles are potential X-ray contrast imaging agents due to their potent X-ray absorption and low toxicity profile, as observed over short durations in animals.[143] A family of low polydispersed dendrimers has been found to be suitable as macromolecular CT contrast agents. As with other macromolecules developed for CT imaging, dendrimers have to be bound to iodinated compounds on surfaces because they are not inherently radio-opaque.[144] Because X-ray CT is far less sensitive than MRI, high concentrations of iodine need to be attached, making chemical synthesis of macromolecular contrast agents challenging. Recently, gold nanoparticles have gained significant attention owing to their biocompatibility and relatively low short-term toxicity.[145-147] In addition, they offer several advantages over conventional iodine-based X-ray CT contrast agents such as higher adsorption coefficient and physical density.[148]

In one study, amine terminated G5 PAMAM dendrimers were used as a nanoplatform to create multifunctional dendrimers for the entrapment of gold nanoparticles probes for X-ray computed tomography.[148] Other study, silver (Ag+) ions have also been coupled with amine-terminated G5 PAMAM dendrimer templates to provide a CT imaging contrast agent with significant potential even though the atomic number of Ag is lower than that of iodine.[149]

2.5.4 Positron emission tomography (PET)

PET is a functional imaging technique that is used to observe the metabolic processes in the body. The system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. Molecular imaging techniques such as PET and Single Photon Emission Computed Tomography (SPECT) utilize, respectively, positron and gamma emitting radionuclides for the generation of signal that allows whole body scan in a single examination. The provided non-invasive evaluation of physiology and pathology merges with external and internal radiotherapy to allow theranostics and personalized medicine. Compared with SPECT, PET is relatively more sensitive and exhibits higher spatial resolution with clinical scanners. The spatial resolution, expressed in millimeters in Figure 3, for both PET and SPECT refers to the minimum distance that the imaging modality can differentiate two independently measured objects.

The PET tracer 2-18F-fluoro-2-deoxy-D-glucose (FDG) is the most widely used tracer in oncology.[150] Another radiotracer, L-methyl-11C-Methionine (11C-methionine), also has been used successfully for PET imaging of brain and lung tumors, non-Hodgkin's lymphoma, breast cancer and head and neck cancer.[150] Of the radionuclides used in clinical practice of PET, Gallium-68 (half-life, $t_{1/2}$ = 68-min) exhibits positron emission intensity >87%.[151] Recently, Ghai, A. et al radiolabeled G4 PAMAM dendrimer with ^{68}Ga via tetraazacyclododecane tetraacetic acid mono (N-hydroxysuccinimide ester) (DOTA-NHS active ester) to provide a conjugate used for observing animal biodistribution and PET imaging.[152]

In a recent report,[153] multifunctional dendrimers were chelated and readily labeled with positron emitting nuclides such as ^{64}Cu. This system serves as a potential platform for PET.

According to the study, N-termini of the dendrimers were modified with aminooxy and conjugated to ketone-bearing LyP-1 and ARAL peptides. Oxime ligation of peptides to dendrimers provided (LyP-1)4- and (ARAL)4-dendrimers conjugated with optical carboxyfluorescein or fluorescein amidite (FAM) and PET probes (6-BAT). The (LyP-1)4-dendrimer-BAT was labeled with ^{64}Cu and enhanced with various targeting peptides to enable a broad range of PET imaging studies for disease detection and assessment of therapeutic delivery.

2.5.5 Single-photon emission computed tomography (SPECT)

SPECT is a nuclear medicine tomographic imaging technique that uses gamma rays and is highly sensitive and quantitative in diagnosis. It enables the assessment of biochemical changes and molecular targets within a living subject.[154] According to a recent report, the high thermal neutron capture cross-section of ^{157}Gd (15.6% natural abundance) of 257,000 barns (the largest value among the known stable radio-isotopes) is useful neutron capture therapy (NCT) for cancers.[155] NCT is mainly associated with tumor-specific delivery systems and involves the production of localized cytotoxic radiations by non-radioactive nuclide delivered to tumor cells. Clinically, technetium-99m (^{99m}Tc) and iodine-131 (^{131}I) are mostly commonly used for SPECT imaging. The synthesis and evaluation of a novel, multimodal blood pool dendrimer-based contrast agent designed for use in preclinical hybrid microSPECT/CT imaging systems with direct applicability to hybrid clinical imaging systems were reported.[156] This multimodal agent offers long intravascular residence time ($t_{1/2}$ = 43 min) and sufficient contrast-to-noise for effective serial intravascular and blood pool imaging by both SPECT and CT. The author summarized that a long-circulating dendrimer system, comprised of triiodinated moieties and chelated Technetium-99m (99mTc), provided effective and simultaneous contrast enhancement in CT and SPECT. Another study reported that a radiolabeled dendrimer-folic acid conjugate

system with 99mTc radionuclide was readily internalized into tumor and is a promising imaging tool for micro-SPECT imaging in live animals.[157]

A recent study reported that the conjugation of chlorotoxin targeted ligand with the amine terminated G5 PAMAM dendrimer labeled with radionuclide ^{131}I for targeted SPECT imaging and radiotherapy of cancer.[158] Most importantly, ^{131}I conferred the dendrimer platform with the ability of targeted SPECT imaging and radiotherapy of a MMP2-overexpressing glioma model in vivo. The authors concluded that the developed radiolabeled multifunctional dendrimeric nanoplatform holds great promise in targeted theranostics of human gliomas.

Recent review also described the synergistic effects of molecular imaging and targeted drug delivery to provide unique opportunities in a relatively new area called image-guided drug delivery' (IGDD).[159] SPECT is the most widely used nuclear imaging modality clinically and is increasingly being applied to targeted therapeutics.[159]

Summary

This review summarized the importance of dendrimers as novel drug carriers and bioimaging agents. PAMAM dendrimers have been used extensively in a variety of biomedical applications, both in the therapeutic and diagnostic, because of there tunable size and non-immunogenicity properties. However, the cellular toxicity is highly depends on the generations, size and surface charges of PAMAM dendrimers. Generally, the cytotoxicity of PAMAM dendrimers can be ranked in the order of hydroxyl-terminated < carboxyl-terminated < amine terminated functional groups. Furthermore, small generation PAMAM dendrimer is the best candidates while, larger generation of PAMAM dendrimer may not be a good choice for biological uses due to its toxicity. Hence, PAMAM dendrimers surface modification bear significant potential as versatile

delivery systems for drugs, antibodies, oligonucleotides, proteins, and peptides as well as diagnostic agents for various imaging modalities in *in vitro* and *in vivo* studies.

Acknowledgments

The authors would like to thank the Ministry of Science and Technology, Taiwan (MOST 103-2221-E-011-035) and the National Taiwan University of Science and Technology (102H451201) for providing financial support.

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