Dendrimers are promising, efficient and smart nanocarriers with applications not only in drug delivery and targeting, but also in diagnosis. Host–guest chemistry, hyperbranched architecture and tailor-made surfaces make numerous surface-engineered dendrimer nanoarchitectures possible.

Dendrimer nanoarchitectures for cancer diagnosis and anticancer drug delivery

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Dendrimers are novel nanoarchitectures with unique properties including a globular 3D shape, a monodispersed unimicellar nature and a nanometric size range. The availability of multiple peripheral functional groups and tunable surface engineering enable the facile modification of the dendrimer surface with different therapeutic drugs, diagnostic agents and targeting ligands. Drug encapsulation, and solubilizing and passive targeting also equally contribute to the therapeutic use of dendrimers. In this review, we highlight recent advances in the delivery of anticancer drugs using dendrimers, as well as other biomedical and diagnostic applications. Taken together, the immense potential and utility of dendrimers are envisaged to have a significant positive impact on the growing arena of drug delivery and targeting.

Introduction

The use of nanotherapeutics for drug delivery and targeting has demonstrated significant potential, particularly in increasing drug safety and reducing drug-associated toxicity to nontarget organs and tissues. Over the past three to four decades, biomedical research has focused on developing nanotechnology for various clinical uses. Dendrimers, in particular, are one of the most promising and efficient nanocarriers for smart drug delivery and diagnostic applications. The chemistry behind dendrimers was first reported in 1978 [1], with the discovery of the first dendrimer reported by Donald A. Tomalia during the early 1980s. Dendrimers are 3D, unimolecular, highly branched monodispersed macromolecules. The word 'dendrimer' originated from the Greek word *dendrons* and its literal meaning is tree or branches, wherein *meros* means parts [2].

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Umesh Gupta, currently an assistant professor in the Department of Pharmacy, Central University of Rajasthan, India, was awarded his PhD in pharmaceutical sciences from Dr Hari Singh Gour Central University, under the mentorship of N.K. Jain. He has previously worked as a research scientist at Ranbaxy Research Laboratories, India and as a postdoctoral research associate at South Dakota State University, USA. He has more than 10 years of research and academic experience. His area of research is mainly focused on dendrimer-mediated drug delivery, solubilization and targeting. Most of his research projects focus on the multiple drug delivery aspects of novel carriers to establish multiapproach treatment strategies. He also has more than 32 publications and six book chapters to his credit.

http://dx.doi.org/10.1016/j.drudis.2016.09.013

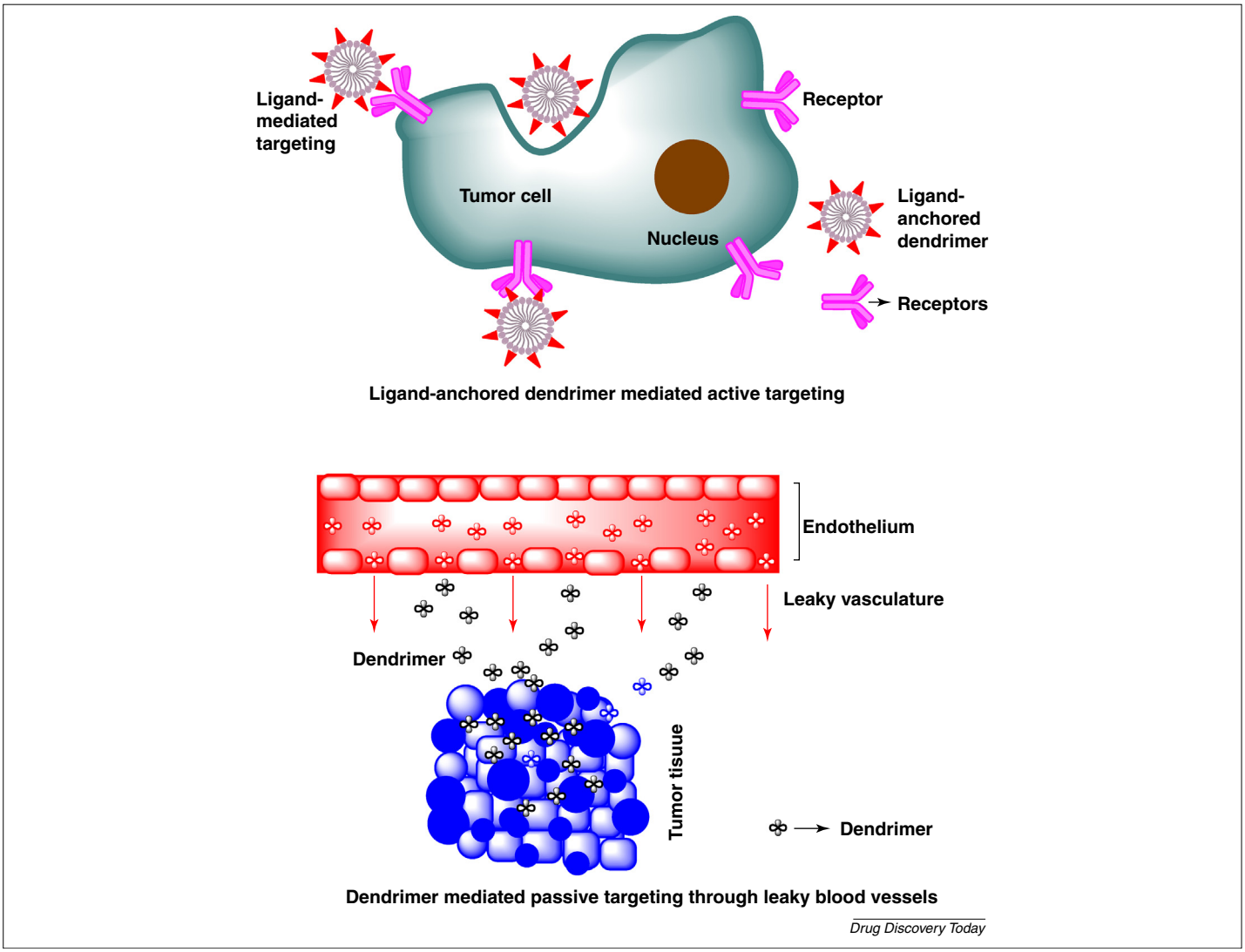

FIGURE 1

Dendrimer-mediated active and passive targeting. Active targeting is usually based on ligand-anchored or conjugated systems that specifically bind to the target site without affecting nontarget sites. Passive targeting can be achieved via the size of the dendrimer used, which affects the enhanced permeation and retention (EPR) effect, leading to leakage that can result in targeting, such as in highly vascularized and leaky blood vessels in solid tumors.

At the same time, Newkome *et al.* [3] reported the synthesis of the same macromolecule and called it 'arborols', originating from the latin word *arbor*, meaning a tree. Typically, dendrimers have an initiator core from which branches emanate and end with terminal surface groups [4]. Dendrimers have been reported to display excellent host–guest chemistry. The exterior surface has multiple water-soluble functional groups that are responsible for the excellent water solubility, whereas the inner core and branches are responsible for the encapsulation of hydrophobic drugs, facilitating enhanced aqueous solubility. Dendrimers significant impact drug solubility as well as drug targeting (active and/or passive targeting; Fig. 1). Dendrimers have been used for the diagnosis of diseases, in particular cancers; thus, here we highlight the diagnostic applications of dendrimers and briefly discuss their ability to deliver anticancer drugs.

The utility of dendrimers for cancer diagnosis

The unique properties of dendrimers, such their monodispersity, modifiable surface functionality and internal cavities, makes them ideal carriers for the targeted delivery (with or without ligand) of therapeutic and diagnostic agents (Fig. 2). The key features of dendrimers that support their use as potential drug delivery agents include their excellent uptake by cells, high density of multiple functional groups like amine, hydroxyl and carboxylic acid, multiple functionalities and their ability to entrap or conjugate relatively higher-molecular-weight drugs at a higher percentage [5,6]. In addition to improving drug solubility and circulation time, dendrimer nanocarriers also facilitate the passive targeting of drugs to tumor tissues through an enhanced permeation and retention effect (EPR). The mechanism behind this targeting results from the increased permeability of the tumor vasculature to certain macromolecules. Compared with other nanocarriers, the unique properties of dendrimers make them ideal candidates for the development of drug delivery systems for the treatment of cancer (Fig. 3) [7]. For example, Kukowska-Latallo *et al.* [8] synthesized PAMAM dendritic polymers <5 nm in diameter as carriers, conjugating them with folic acid and methotrexate (MTX) for tumor targeting. On the basis of confocal microscopy observations, the authors

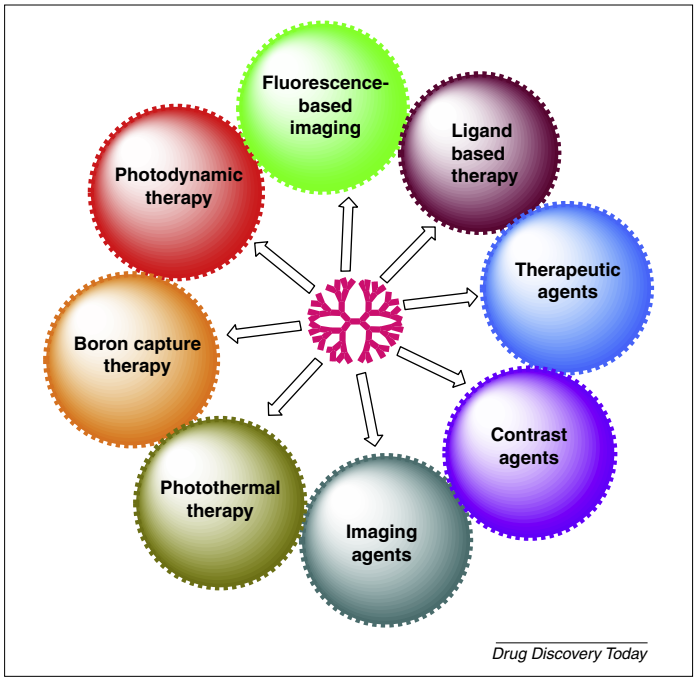

FIGURE 2

Schematic representation of the diagnostic and biomedical applications of dendrimers. The versatile nature of dendrimers not only finds applications in drug delivery and targeting, but also has equal impact in diagnostic applications, such as contrast agents, in phototherapy, as well as in imaging.

suggested that the targeting carrier (<50 nm) was able to escape the vasculature via the EPR effect and interact with the individual tumor cells.

Advances in nanotechnology have enabled the development of different nanomaterial-like dendrimers that can be used as diagnostic aids in diverse molecular-imaging applications. In particular, diagnostic applications, including as magnetic resonance imaging (MRI) contrast agents in boron neutron capture therapy have also been reported, as we discuss below.

MR imaging

Imaging techniques are used in cancer therapy to diagnose and identify the location of disease sites as well as to plan treatment, subsequent to their identification. Computed tomography (CT) and MRI are well-established methods of imaging associated with cancer diagnosis [9]. MRI is a non-invasive technique for the diagnosing tumors in soft tissues. In addition to therapeutic agents, various MRI agents have also been delivered using dendrimers. For example, Langereis *et al.* [10] reported the synthesis of 5–6-nm gadolinium-diethylene triamine penta-acetic acid (Gd-DTPA)-terminated poly(propylene imine) (PPI) dendrimers as MRI agents. Transmission electron microscopy (TEM) showed that these dendrimers did not self-aggregate. The efficiency of these dendritic contrast agents in MRI, expressed in terms of longitudinal (r^1) and transverse (r^2) relaxivities, was measured at 20°C and 1.5T. The value of r^1 and r^2 has been increased with increasing generation number of dendritic system. The reported r^1 and r^2 value of G0 and G5 were 8.2 ± 0.2 and 19.7 ± 0.3, 9.6 ± 0.2 and 27.8 ± 0.3, respectively. Talanov *et al.* [11] reported a PAMAM dendrimer-based nanoprobe with dual MR and fluorescence (Fl) modalities. Gd (III) was covalently attached to a dendrimer to create a fresh macromolecular contrast MRI agent. The authors used 2-(4-isothiocyanatobenzyl)-6-methyl diethylenetriaminepentaacetic acid (1B4M-DTPA) and Cy5.5 as a bifunctional chelating agent. The PAMAM dendrimers covalently attached with the Gd (III)-DTPA chelates and units of the near-infrared (NIR) fluorescent dye, Cy5.5, to form a dual-modality MRI-FI agent. The authors prepared nine different batches and selected one for the evaluation that comprised 1.25 molecules of Cy dye and 145 molecules of 1B4M-Gd. Thus, this dual-labeled dendrimer contrast agent enables MRI and FI to be performed simultaneously. Saad *et al.* [12] compared the use of linear polymers, liposomes and dendrimers for tumor-targeting treatment and imaging. They used star-like PAMAM dendrimers for the delivery of paclitaxel and/or Cy5.5. LHRH was used as the targeting moiety because LHRH receptors are overexpressed in the plasma membrane of cancer cells.

Zhu *et al.* [13] prepared hexameric Mn(II) dendrimers as MRI contrast agents. The dendrimers comprised six tyrosine-derived $[Mn(EDTA)(H_2O)]^{(2-)}$ moieties coupled to a cyclotriphosphazene core. Variable temperature (17) O NMR spectroscopy revealed a single water co-ligand per Mn(II) that under went fast water exchange [kex = $(3.0 \pm 0.1) \times 10^8$ s^{-1} at 37°C]. The 37°C per Mn(II) relaxivity ranged from 8.2 to 3.8 mM^{-1} s^{-1} from 0.47 to 11.7 T, which is sixfold higher on a per molecule basis compared with Gd based contrast agent. The authors reported that Mn(II)-containing dendrimers represent a potential alternative to Gd-based contrast agents, especially in patients with chronic kidney disease, where the use of current Gd-based agents can be because of fast blood clearance and elimination via both the renal and hepatobiliary routes. Similarly, Niidome *et al.* [14] constructed a contrast agent by combining PROXYL groups that had nitroxyl radicals with PEG-modified dendritic poly(L-lysine), which accumulate in tumors via the EPR effect. PROXYL groups at the PEG chain termini on KG6 were advantageous in Overhauser enhanced (O)MRI, because the ESR signal of the nitroxyl radical was maintained without decay caused by mobility restriction, even if the PROXYL groups were attached at 25 mol% on one molecule. After intramuscular injection of the molecule modified at 25 mol% (PR25-PEG-KG6), a significant OMRI signal was observed at the injected site. However, no signal was detected in the tumor after intravenous injection of PR25-PEG-KG6 into a tumor-bearing mouse, although PR25-PEG-KG6 itself accumulated in the tumor.

Contrast agents

The surface of dendrimers can be functionalized with multiple targeting ligands to provide enhanced cellular uptake via polyvalent binding as well as to improve the imaging specificity [15]. To confirm polyvalency, Licata *et al.* [16] proposed a lock-and-key theoretical model comprising dendrimers conjugated with 'keys', that is, folic acid, which 'locks' or binds to particular cell membrane proteins expressing folate receptor. The increased concentration of 'locks' on the surface led to extended residence time for the dendrimer and resulted in greater incorporation into the cell. Thus, this cooperative binding ultimately leads to the enhancement of cell specificity.

These modifications of dendrimers makes them suitable carriers for targeting approaches as well for developing nanoscale macromolecular contrast agents to overcome the drawbacks of conventional small-molecular compounds [17]. Dendrimers can be linked

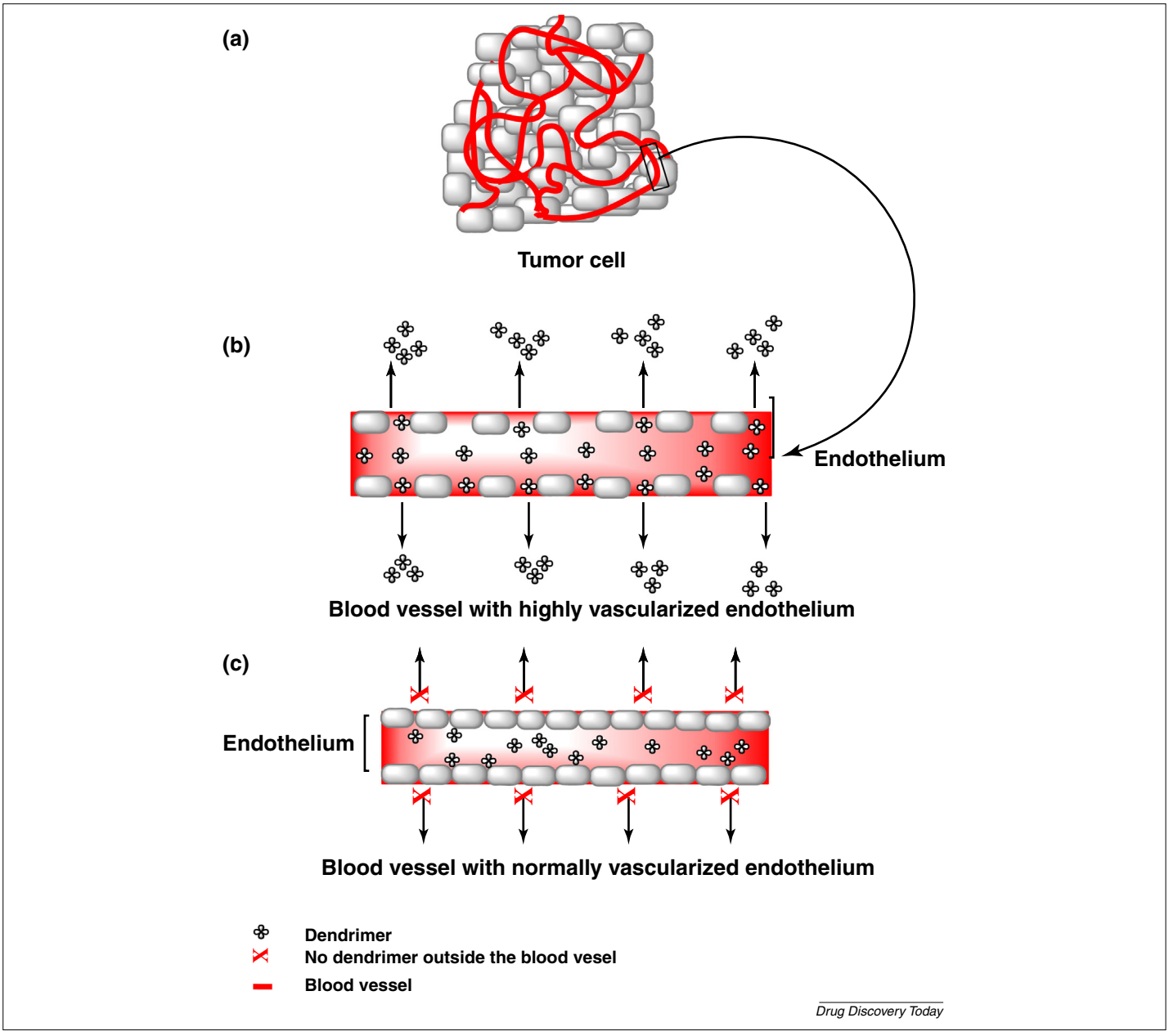

FIGURE 3

Dendrimer-mediated enhanced permeability and retention (EPR) effect. The EPR effect enables nanocarriers to accumulate and be retained by tumor tissues. Dendrimers that are circulating in the blood can accumulate in a highly vascularized tumor by extravasation through disorganized or leaky blood vessels at the tumor site. Generally, the passive targeting approach is based on the EPR effect.

with fluorescent molecules or iodinated small-molecular CT contrast agents. The unique structure and varying generation of dendrimers allows the development of bimodal or multimodal contrast agents for (dual mode or multimodal) imaging applications with improved diagnosis accuracy [18–21].

Contrast agents based on FI

FI can provide better stability, high specificity and biocompatibility coupled with excellent resolution without exposure to ionizing radiation. These agents also provide unique advantages over other imaging modalities in terms of their sensitivity, precise detection capabilities and cost-effectiveness. Dendrimers can be useful for diagnostic purposes because of their functionality as dual-modality contrast agents, such as MR/fluorescence contrast agents and MR/CT contrast agents. Dual-modality agents have been recently reported that could improve the accuracy of a diagnosis when a single imaging modality fails to provide all the necessary insights [22]. For example, hydroxyl-terminated PAMAM dendrimers were able to generate blue fluorescence with high quantum yield via a simple oxidation process [23]. Wang and Imae [24] also reported that both PAMAM and PPI dendrimers display fluorescence emission under acidic conditions. Recently, Tsai *et al.* [25] showed that intrinsically fluorescent PAMAM dendrimers could be used as gene vectors and nanoprobes for nucleic acid delivery. These studies focused on exploiting the fluorescence properties of dendrimers for different cellular-imaging applications. Future studies would need

to further demonstrate the usefulness of PAMAM and PPI dendrimers for disease diagnostics using fluorescence-based imaging probes.

X-ray and CT imaging contrast agents

X-ray and CT are well-established techniques that can be used to visualize tissues, organs, or blood vessels with enhanced contrast and desirable image quality by using stable contrast agents [26]. Dendrimers have been used as nanocarrier systems to load or prepare different types of CT contrast agent. The so-formed dendrimer-based CT contrast agents are nanoscopic in size, are able to overcome the drawbacks of small-molecular iodinated contrast agents, and can be used for CT imaging of different biological systems [27]. Despite the small number of published studies in this area, it holds significant research potential.

Radionuclide-based contrast agents

Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are the most common and extensively used imaging techniques in nuclear medicine imaging. For these studies, radionuclide- or radiopharmaceutical-based contrast agents are generally required for effective imaging [28]. Given the drawbacks of the small-molecular radionuclide complexes or agents, which can be rapidly cleared or metabolized from organs and/or are unable to accumulate sufficiently in the targeted tissue (such as tumor), other nanoparticle (NP)- or macromolecule-based approaches are desirable [29]. Various radionuclides have been labeled using dendrimer scaffolds, resulting in agents with desired functionality for enhanced and specific imaging of particular diseased tissues [30].

Photodynamic and photothermal therapy

Photodynamic therapy (PDT) is a treatment that uses photosensitizers or photosensitizing agents as a drug along with particular types of light (typically a laser source). When photosensitizers are exposed to a specific wavelength of light, they produce reactive oxygen species (ROS) that kill nearby cells (Fig. 4). PDT, which uses functionalized NPs that can selectively localize to the diseased tissues or cells, is a promising method for the precise treatment of particular disease sites. The action of PDT comprises the selective attachment of photosensitizing molecules (PSs) to the diseased tissue. Irradiation of tissues at specific wavelengths of light activates the PSs, thus producing ROS and promoting cell death [31]. Aoki *et al.* [32] reported the improved photosensitivity of dendrimers by converting surface terminal dendritic acrylate into polyallyl to form a thiol-ene system. Thiol-ene photopolymers have high ultraviolet (UV) curability of their thin film even under aerobic conditions. The authors reported the photosensitivity of these dendrimers in the form of their pencil hardness, which was used to monitor the hardening of UV exposed films. The sensitivity curve of polymers was also constructed by measuring the pencil hardness. UV exposure was reduced because of the increase in number of relative allyl groups that are attacked by the thiyl radicals and, additionally, by the increased mobility of thiyl radicals in the film. Kojima *et al.* [33] prepared nanocapsules of photosensitizers including rose bengal (RB) and protoporphyrin IX (PpIX) by using PEGYlated dendrimers for use in PDT. The authors synthesized two PEG-attached dendrimers, the PAMAM dendrimer of G4 and the PPI dendrimer of G5, each with 64 termini, resulting in dendrimers named PEG-PAMAM and PEG-PPI.

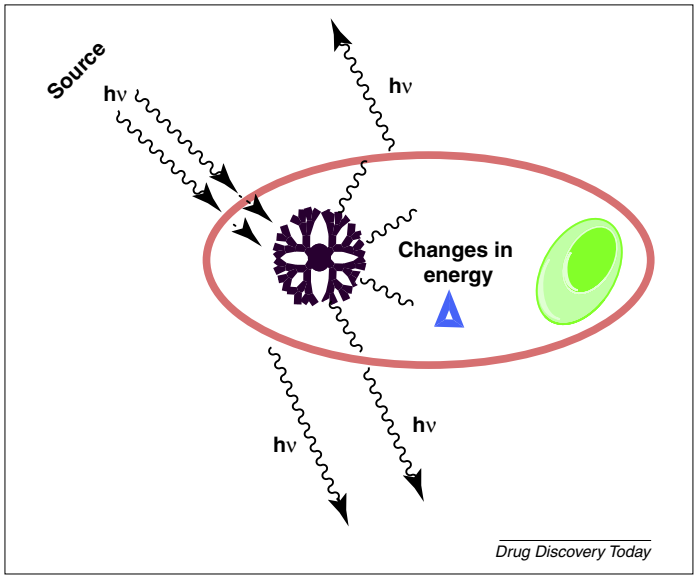

FIGURE 4

Applicability of dendrimers in photothermal therapy and the changes in the energy state as a result of photon emission. The multiple surface functionality of dendrimers uniquely contributes to the photo-emission process.

The results showed that fewer PpIX molecules were encapsulated by both PEG-attached dendrimers and were more stable under physiological conditions compared with free RB. Similarly, Spyropoulos-Antonakakis *et al.* [34] prepared a combination of PAMAM and PAMAM/ZnPc nanodrugs for use as PDT for the effective treatment of atherosclerosis. Using atomic force microscopy (AFM), The authors investigated the aggregation of PAMAM (G0) as a drug delivery carrier, as well as the ability of conjugated G0 PAMAM dendrimers with a ZnPc photosensitizer, to target symptomatic and asymptomatic human carotid tissues. Addition of G0 PAMAM or G0/ZnPc PAMAM conjugate to the carotid tissues, either healthy or atheromatous, significantly changes the texture characteristic of human carotid with varying agglomerating responses of the NPs. The resultant had an inverse impact to healthy tissue compared with deposition on atheromatous one with different aggregation features.

PDT has emerged as a gold standard minimally invasive tumor therapy. The first methods for preparing metal-encapsulated dendrimers for application in the biomedical field were reported during the past decade. Such dendrimers had the added advantage of enabling control for the fine tuning of the biological interactions elicited by the metal particles, including improved biocompatibility, retention and ease of surface modification, resulting in their potential use as biomarkers, contrast agents and in PDT [35].

Boron neutron capture therapy

The principle of boron neutron capture therapy (BNCT) is based on a lethal $^{10}B(n,\alpha)^{7}Li$ capture reaction that occurs when ^{10}B is irradiated with low-energy thermal neutrons to produce high energy α-particles and ^{7}Li nuclei. These particles have limited path lengths in tissue (b^{10} mm) and, therefore, the resultant toxicity is limited to cells that have internalized ^{10}B [36]. PAMAM has been used for investigating intratumoral delivery of BNCT agents. Certain human gliomas have been targeted with boronated G5-PAMAM conjugated to anti-EGF receptor monoclonal antibodies, which act

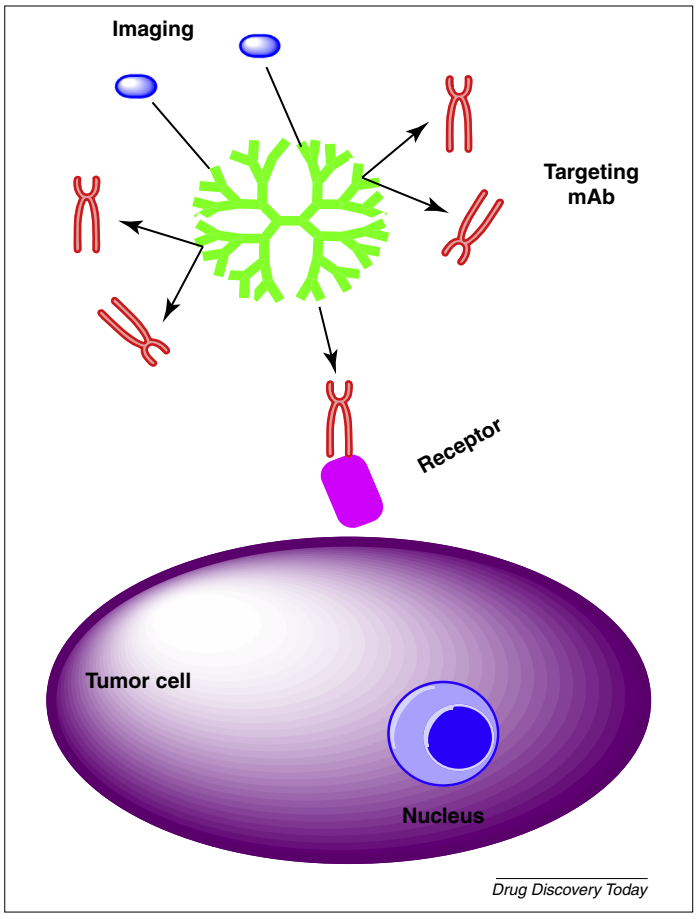

FIGURE 5

The active targeting approach used for diagnosis and treatment via monoclonal antibody (MAb) and imaging agents containing dendrimer conjugates. Compared with other methods, the conjugation at the tailor-made surface of dendrimers has superior attributes and enhances effective receptor-mediated targeting.

against overexpressed tumor cell receptors. In this study, Wu *et al.* reported the conjugation of dendrimers with cetuximab (Ctx), an EGF receptor-specific monoclonal antibody (Fig. 5), and then evaluated its effects *in vitro* and *in vivo* using F98 cells. The cell-binding capability of the cetuximab-conjugated dendrimers was comparable to that of free cetuximab *in vitro*. Intratumoral injections in mutated rats showed a 13.8-fold increase in tumor boron content for the targeted dendrimers over unmodified boronated G5-PAMAM [37]. In another study, Wu and coworkers [38] examined the uses of Ctx-G5-B_{1100} conjugates with or without boronophenylalanine (BPA) or sodium borocaptate (BSH), two of the drugs most currently used for BNCT, for the treatment of $F98_{EGFR}$ glioma. Boronated dendrimers were delivered either through a positive-pressure method (convection enhanced delivery, CED) that assists transport across the blood–brain barrier (BBB), or intratumorally resultant in high retention of boron in the gliomas, with around 50% more accumulation resulting from the CED method. Backer *et al.* designed boronated G5- PAMAM (BD) with vascular endothelial growth factor (VEGF) and near-IR Cy5 dye, or VEGF-BD/Cy5, for targeting upregulated VEGF receptors overexpressed in tumor neovasculatures. The result of near-IR imaging confirmed the accumulation and increased concentration at the tumor site [39].

Exclusive dendrimer types

As per the reported literature, a variety of dendrimers has been used for encapsulation or conjugation of different types of anticancer drug with or without ligands. Here, we discuss examples of such dendrimers.

PAMAM-PEG dendrimers

Cationic toxicities and biological fates associated with dendrimers have been major concerns relating to their use in the clinic. Researchers have tried to neutralize this cationic charge-associated toxicity through surface modifications using biocompatible polymers such as PEG. Surface engineering of dendrimers has also been performed for other purposes, such as to achieve targeting of drugs and bio-actives to the desired site on the body [40]. She *et al.* [41] used PAMAM dendrimers with poly(ethylene glycol) (PEG) grafts that could encapsulate drugs in the core. The PEG surface provided the necessary shielding of the cationic surface charge, resulting in a biologically safe carrier.

Glycodendrimers

Investigations of novel carbohydrate-oriented biological interactions led to a new strategy of rational drug design for diagnostics and therapeutics. Glycodendrimers are well-defined monodispersed macromolecules that have a carbohydrate moiety in the structure of the dendrimer [42]. Vannucci *et al.* [43] reported a *N*-acetyl-glucosamine-coated glycodendrimer and its impact on anticancer immunomodulation strategy via a carbohydrate-triggered immune response.

Poly L-lysine dendrimers

Among the several attempts to reduce the toxicity associated with dendrimers, many groups have attempted to synthesize new dendrimers using amino acids (e.g. lysine [44]). Jain *et al.* [45] reported a PPI dendrimer for selective targeting of doxorubicin (DOX) conjugated with folate ligand. Authors of this study reported drug selective targeting and anti-angiogenic effects for anticancer agents. The drug release was noted to be higher under acidic pH. The dendrimers developed were highly biocompatible with low toxicity. Several other lysine-based dendrimers have since been reported that have promising properties compared with other classes in terms of safety, efficacy and toxicity.

Triazine dendrimers

The unique structure of dendrimers provides the opportunity to design dendrimers using any type of multifunctional core or branching units. Researchers have used several small molecules as the core for the synthesis of newer types of dendrimer, such as diaminobutane (DAB), ethylene diamine (EDA), ammonia and citric acid (CA). Triazine is a synthetic moiety with three junctures that has been used as a core for dendrimers by some research groups, such as for using in caging guest molecules [46]. Lim and Simanek [47] reported the synthesis of water-soluble triazine dendrimers PEGylated and then loaded with paclitaxel (PTX). The authors synthesized two variants named 4a and 4b. PEGylated dendrimer 4a was 30 wt% PTX, 52 wt% PEG and 18 wt% dendrimer, whereas 4b was 18 wt% PTX, 71 wt% PEG and 11 wt% dendrimer.

Carbosilane dendrimers

The strength of carbosilane dendrimers (CBD) is related to adequate energy of the C–Si bond along with its low polarity. These properties can be modified by functionalization of the periphery with polar moieties to make the dendrimer hydrophilic [48]. The 1,3,5-trihydroxybenzene core leads to CBD that are less congested than dendrimers with a silicon atom core. CBDs have been successfully used as nonviral vectors for transfecting different types of nucleic acid in many diseases, such as AIDS and cancer [49].

Phosphorus dendrimers

Phosphorus dendrimers are important for biomedical engineering research because of the presence of phosphorus, which is an essential element in living organisms. This unique class of dendrimer reflects their positive effect on the growth of neuronal cells and natural killer cells, and their use as delivery platforms for ocular bioactive agents as well transfection and imaging agents. Phosphorus-containing dendrimers also have potential utility as highly sensitive biosensors [50]. The presence of a hydrophilic surface and a hydrophobic backbone facilitates their efficient penetration of biological membranes. Recently, a study revealed the interaction of a phosphorous-containing dendrimer with a model membrane and its effects on the phase behavior of lipid vehicles as well as its physical properties, such as the surface charge of membranes [51].

PAMAMOS

PAMAMOS dendrimers benefit from the combined properties of hydrophilic PAMAM interiors and reactive hydrophobic organosilicon (OS) exteriors. Depending upon the exact composition and generation of the PAMAMOS dendrimers, and the precise composition and set of the reaction mixture selected, a network structure can be obtained that is a plastomeric or elastomeric film, coating, or other feature containing hydrophilic and hydrophobic PAMAM and organosilicon OS domains [52].

Functionalized/ligand-anchored dendrimers in drug delivery

The delivery of drugs to the desired site in the body is a main focus of biomedical research. In this regard, ligand-based targeting is most important for achieving the optimum delivery of drug to a particular site. Ligands are responsible for the targeted delivery of the drug to the tumor cells. Ligands that have been reported as being used with dendrimers to this end are shown in Table 1. They include folic acid (which is overexpressed in many epithelial cancer cells, including kidney, lung, breast, ovary and neck cancers [53]), biotin (its levels are high in cancerous cells compared with normal cells [54]), *N*-acetyl-glucosamine [55] and riboflavin (a molecule essential for the biosynthesis of flavin-based redox cofactors, such as FMN and FAD [56]). Other surface-engineering/functionalization strategies have been reported that have additional advantages; for example, acetylated polypropylene dendrimers were reported to hide the surface cationic charge or reduce the hemolytic toxicity [57]; amino acid-functionalized dendrimers were reported to improve the gene transfection efficiency and to achieve reduced toxicity in primary neuronal cells [58]; and arginine-grafted PAMAM dendrimers were reported to increase the gene delivery and transfection efficiencies [59].

TABLE 1

Ligand-based targeted drug delivery using dendrimers[a]

Ligand	Anticancer drug	Dendrimer type	Refs
Biotin	CDDP	PAMAM	[78]
Folic acid	MTX	PAMAM	[67,68]
D-Glucosamine		Polyether-co-polyester	[65]
Riboflavin	N/A	PAMAM	[56]
N/A	5-FU	PAMAM	[74]
WGA,Tf	DOX	PAMAM	[88]
Trastuzumab	N/A	KG6E	[94]

[a] The table details drugs delivered using dendrimers in terms of the ligands conjugated to the dendrimer surface.

Biosafety issues and challenges

Biosafety relates to the prevention of significant loss of biological integrity, in relation to both ecological impacts and human health. Toxicity issues, such as the cytotoxicity, hemolytic, hematological toxicity, immunogenicity and in vivo toxicity of dendrimers are generally associated with the cationic charge of the terminal-NH_2 groups on the periphery of the dendrimer. Thus, in terms of dendrimers, the effects of their generation number and relevant concentration on humans are important. Luo *et al.* [60] presented the use of peptide dendrimers as efficient and biocompatible gene delivery vectors. In a gel retardation assay, the dendrimers formed complexes with plasmid DNAs (pDNAs), evident from the inhibition of the migration of pDNA at N:P ratios of 0.5, 1 and 2 by G3, G4 and G5 dendritic generations, respectively. AFM revealed that all four generations of dendrimer/DNA complexes studied had similar particle sizes (100–200 nm). Zeta potential measurements showed that, as the N:P ratio increased from 1 to 25, the higher generations of peptide dendrimers tended to produce increased positive potentials, demonstrating the stronger potency of the complexes to interact with negatively charged cell membranes. Zeta potential measurements showed that, as the N:P ratio increased from 1 to 25, resultant zeta potential changed from negative to positive for dendrimer/pDNA complexes. The higher generations of peptide dendrimers tended to produce increased positive potentials, demonstrating the stronger potency of the complexes to interact with negatively charged cell membranes. In vitro gene transfection and in vivo cytotoxicity investigations revealed good biocompatibility of the dendrimers and their complexes over the different N:P ratios studied. In another study, Luo *et al.* [61] also formulated multifunctional gadolinium-based peptide dendritic macromolecules as liver-targeting imaging probes using galactose moieties as the macromolecular scaffold. In this study, the authors used generations 2, 3 and 4 that had highly controlled structures and single molecular weights. The study revealed that Gd-ligand agents exhibited up to threefold increases in T_1 relaxivity compared with Gd-DTPA complexes. As a part of their research, the authors used galactose as a targeting agent and found a higher cell uptake *in vitro* as demonstrated in a T_1-weighted scan.

Despite the utility seen *in vitro*, several challenges remain in terms of the use of dendrimers *in vivo* in the clinic. In addition, multifunctional nanocarriers based on the dendrimeric system remain a challenging because of the complexity of conjugating

numerous types of molecule to a dendrimer. This conjugation can change the physicochemical properties and other biological activities of the dendrimer. It is difficult to formulate dendrimers on a large scale because of purity and cost escalation. Additional modification of the synthetic steps required to synthesize new dendrimers need to be tested so that they maintain their bioactivity [62]. Toxicity issues, such as the cytotoxicity, hemolytic and hematological toxicity, immunogenicity and *in vivo* toxicity, of dendrimers are generally associated with the cationic charge and the terminal-NH_2 groups on the periphery of the dendrimer. These hurdles thus limit their clinical application. The toxicity profiles also generally depend on the generation number and concentration of dendrimer used. Published studies provide information that can be used to develop new dendrimers that avoid some of these issues. Various approaches have been proposed by different research groups to reduce the toxicity associated with dendrimers through modification of the parent dendrimer as drug delivery vehicles. The list of biodegradable functional groups proposed includes polyether, polyether imine, polyester, polyether–copolyester (PEPE), phosphate dendrimers, citric acid, triazine, melamine and peptide-based dendrimers [63].

Anticancer drug delivery

Dendrimers has been widely used for the delivery of anticancer drugs by active and passive mechanisms (Table 2). Here, we provide a brief discussion of the use of dendrimers as drug delivery vehicles.

TABLE 2

Anticancer drugs delivered using dendrimers[a]

Anticancer drug	Dendrimer type	Refs
5-FU	PAMAM-mPEG-PDEA	[73]
	PAMAM	[74]
	Polyglycerol	[75]
CDDP	PAMAM	[77]
	PAMAM-COOH	[78]
MTX	Polyether-co-polyester	[65]
	PAMAM	[66–68]
PTX	Polyglycerol	[80]
	PAMAM	[12,79,81,84]
	Triazine	[47]
	PPI	[82]
	PAMAM, PEGylated-PPI	[87]
	PAMAM, PEGylated-PPI, FA-DLSP-PLLA	[89]
DOX	PEGylated-PAMAM,	[86]
	RGD modified PAMAM	[87]
	PAMAM-PEG-WGA-Tf	[88]
	Polyester	[95]
	PAMAM	[97,98]
Camptothecin	PAMAM	[92]
Adriamycin	PAMAM	[97]
Dimethoxycurcumin		[98]
Tamoxifen		[92]

[a] Dendrimers have been used for the delivery of several categories of anticancer drugs. As shown above, the building blocks used are not limited to PAMAM.

Methotrexate

MTX is an antimetabolite and folate antagonist drug widely used in the treatment of various cancers, autoimmune diseases and ectopic pregnancy, and for the induction of medical abortions [8]. MTX was one of the first anticancer drugs reported to be delivered using dendrimers. Dongen *et al.* [64] synthesized and characterized generation-5 PAMAM dendrimer–MTX conjugates by utilizing G5-$(COGMTX)_n$ and G5-$(MFCO\text{-}MTX)_n$, as molecular linkers. In addition to above conjugate, G5–G5 (D) dimer, D-$(COGMTX)_n$ and D-$(MFCO\text{-}MTX)_n$ were also prepared. The monomer G5-$(COG\text{-}MTX)_n$ conjugates showed weak and rapidly reversible binding to folate-binding protein (FBP) similar to that seen with monovalent MTX binding. The next conjugation, D-$(COG\text{-}MTX)_n$, exhibited a slow onset, tight-binding mechanism in which the MTX first bound to the FBP, inducing protein structural rearrangement. The concentration of MTX directly affects the extent of irreversible binding. Dhanikula *et al.* [65] reported the polyether-co-polyester dendrimer-based delivery of MTX for enhanced efficacy and transport capability in gliomas. The authors used D-glucosamine as a ligand for the selective targeting and higher BBB permeability because glucose receptors, such as the GLUT-1 receptor, are overexpressed in brain tumors, stomach, liver, pancreas, colon, lungs and retina. The glucosylation cellular uptake was increased up to 2–4.5-fold compared with free MTX. The dendrimer use reduced the MTX resistance and the IC_{50} value was also reduced 9–15 times. The authors also reported that the drug combined with the dendrimer had higher potency than free drug and these MTX-loaded dendrimers were able to kill MTX-resistant tumor cells. Similarly, Zhang *et al.* [66] synthesized saccharide-terminated G3 PAMAM dendrimers for MTX. The authors developed polyvalent G3-MTX-FI conjugates that contained the imaging agent fluorescein-5(6)-carboxamido hexanoic acid (FI) and reported a folate receptor-based uptake of conjugates. The cytotoxicity was similar to that seen with MTX at higher doses. Zhang *et al.* suggested that this might be due to the release of MTX from the conjugate following endocytosis and its transit through the acidic endosome/lysosome compartments, although no evidence in support of this hypothesis was provided.

Huang *et al.* [67] reported a facile conjugation of MTX (cytotoxic drug), folic acid (targeting agent) and fluorescein (imaging agent) with G3 PAMAM dendrimers using copper-free click chemistry. The authors reported the resulting surface modification by cyclooctyne groups. The platform was then clicked with γ-azido functionalized MTX. The yield of the complex was 90%. The copper-free click reactions were efficient and had high yields. Castro *et al.* [68] reported one-step synthesized PAMAM frame-based targeted nanodevices for MTX. The authors prepared four different water soluble conjugates named F1–F4 with or without folic acid. They selected F3 and F4 for biological testing in which F3 was lacking folic acid, whereas F4 included FA. The resulting anticancer activity was enhanced in cancer cells in human lymphocyte cells and growth inhibition was decreased by 80%. Patri *et al.* [69] described how drug delivery can be achieved by coupling a drug to a polymer either via a hydrophobic drug complexed within the hydrophobic dendrimer interior to make it water soluble or by covalent coupling onto the surface of the dendrimers. The authors investigated the toxicity and solubility profile of dendrimers with surface hydroxyl groups and targeting studies were reported using folic acid as the targeting ligand. Zhang *et al.* [70] synthesized and characterized an enzyme-responsive mPEGylated peptide

dendrimer–GFLG–doxorubicin conjugate (dendrimer–GFLG–DOX) as a nanoscale drug delivery vehicle for the effective management of ovarian cancer therapy via a two-step highly efficient copper-catalyzed alkyne–azide click cycloaddition (CuAAC) reaction. The above formulation was characterized by dynamic light scattering (DLS) and TEM. Compared with free drug, the dendrimer–GFLG–DOX conjugate-based nanocarrier system exhibited higher accumulation and retention within SKOV-3 ovarian tumor tissue. No obvious systemic toxicity was observed with the mPEGylated peptide dendrimer–DOX conjugates, proving this strategy promising for the treatment of ovarian cancer. Li *et al.* [71] prepared and characterized a peptide dendritic copolymer–doxorubicin conjugate that was amphiphilic in nature and self-assembled to become an enzyme-responsive anticancer agent through a two-step efficient click reaction. The results of both studies, based on DLS and TEM, confirmed the self-assembly into dense NPs with a negatively charged surface of the mPEGylated dendron–GFLG–DOX conjugate. These NP-based preparations were shown to efficiently kill cancer cells *in vitro*. FI confirmed the long-term accumulation and retention of NPs within the tumor.

She *et al.* [72] synthesized conjugates of dendronized heparin DOX as a pH-responsive drug delivery system for cancer therapy. They further characterized this formulation via DLS and TEM. The 9.0 wt% of DOX exhibited pH-sensitive properties because of the quick drug release rate at pH 5.0 and the slower rate at pH 7.4. The NPs showed strong antitumor activity and high antiangiogenic effects, and induced apoptosis in the 4T1 breast tumor model.

5-Flurouracil

5-Flurouracil (FU) is used to treat several types of cancer, including colon, rectum, and head and neck cancers. Many research groups have attempted to deliver 5-FU using different nanocarriers, such as polymeric micelles, NPs and dendrimers. Jin *et al.* [73] reported a pH-responsive dendrimer for tumor-targeted delivery using surface-modified 4.0G PAMAM dendrimers. The surface of the dendrimer was modified by mPEG and the core was poly [2-(*N*,*N*-diethyl amino) ethyl methacrylate] (PDEA). PEG enhanced the circulation of the drug and PDEA was responsible for its pH responsiveness. The authors reported that the release of the drug was pH responsive and that it was higher (100%) at pH 6 than at physiological pH 7.4 over 6 hours. They also reported increased anticancer activity against H_{22} cells because of the quicker release and targeted delivery of the drug resulting from the lower pH at the tumor site. Buczkowski *et al.* [74] studied the solubility of 5-FU enhanced by PAMAM dendrimers. There was a linear relation between the solubility and dendrimer concentration in the range 2.5–50 mM. The authors concluded that the drug molecules not only were bound to the active sites at the surface, but also occupied vacant space in the dendrimer. Lee and Ooya [75] reported an interaction between 5-FU and polyglycerol dendrimers (PGD) confirmed by ^{19}F NMR and ^{1}H NMR. The authors found that the drug molecules occupied the vacant space of the dendrimer in G1 and G2, whereas there were interactions in the G3 dendrimer because of the change from the diketo form of 5-FU to its keto-enol form.

Cisplatin

Cisplatin (CDDP) was the first US Food and Drug Administration (FDA)-approved metallic (platinum) coordination compound and chemotherapeutic drug; it is used to treat various cancers, including testicular, ovarian, bladder, head and neck, and non-small cell lung cancers [76]. Dendrimers have also been reported for use in the delivery of this drug. Kirkpatrick *et al.* [77] designed different half-generation PAMAM dendrimers as active carriers for CDDP. The drug loading and release behavior were found to depend on dendrimer size. It was observed that, as the size of the half-generation dendrimer increased, both drug loading and release also increased. The anticancer activity of dendrimer–CDDP conjugates was investigated against the A2780, A2780cis and A2780cp ovarian cancer cell lines and in *in vivo* studies against A2780 xenografts. Yellepeddi *et al.* [78] prepared biotinylated PAMAM dendrimers for the intracellular delivery of CDDP to ovarian cancer cells. The authors used PAMAM G_4NH_2 and PAMAM $G_{3.5}COOH$ in which 22 and 19 biotin molecules were attached to dendrimers, respectively, by biotinylation; the resulting conjugates were in the size range 20–40 nm and encapsulation of drug ranged from 5.33% to 21.10%. The authors found that biotinylated $PAMAMG_4NH_2$ showed the highest loading of CDDP, whereas biotinylated $PAMAMG_{3.5}$ COOH showed the lowest (21.10 ± 0.453 and 5.33 ± 0.975, respectively). IC_{50} values of dendrimer-CDDP complexes were significantly lower than that of free CDDP in the OVCAR-3, SKOV-3 and CP70 cell lines.

Paclitaxel

PTX is a diterpenoidal taxol anticancer drug used to treat breast and ovarian cancers. It is obtained from the bark of the pacific yew tree *Taxus brevifolia*. The drug promotes tubulin polymerization by forming a hyperstabilized structure, disrupting the normal tubule dynamics essential in cellular division, thereby inducting cell death [79]. However, PTX is a hydrophobic drug and several attempts have been made to enhance its water solubility using dendrimers, such as delivering it via different nanocarriers, including dendrimers. Ooya *et al.* [80] studied the water solubility of PTX with polyglycerol dendrimers and found that solubility of the drug was associated with the generation of dendrimer and was higher than PEG-400 even at a very low dendrimer concentration (10 wt%), which was probably because of the dendritic structure. Khandare *et al.* [79] reported dual conjugation with PTX, one with linear *bis*-PEG and another with a PAMAM G4 hydroxyl-terminated dendrimer. The authors reported that the *in vitro* cytotoxic activity of the conjugations against A2780 human ovarian cancer cells was ten times more for the PTX-dendrimer–succinic acid conjugation, but 25 times less with the linear PEG–PTX conjugate. Teow *et al.* [81] studied the PAMAM G3 dendrimer-based delivery of PTX as a way of overcoming cellular barriers. The authors grafted lauryl chains to the surface of the dendrimer as a permeability enhancer, and glutaric anhydride as a linker. Free G3 PAMAM dendrimers did not show any cytotoxicity, whereas the lauryl chain-containing G3 PAMAM dendrimers showed cytotoxicity against Caco-2 cells and primary cultured porcine brain endothelial cells (PBECs).

Jain *et al.* [82] investigated the targeting potential of 4.5G of PPI dendrimers grafted with the primary monoclonal antibody mAkB1. The immunodendrimer showed higher specificity toward mesothelin (a protein overexpressed in tumor cells)-containing cells (OVCAR-3) but less specificity toward A-431 cells (mesothelin-negative). The delivery of PTX using a range of dendrimers has

been reported, and this focus is likely to reflect the widespread use of this drug in the treatment of many types of cancer.

Doxorubicin

DOX is an anticancer drug widely used in the treatment of many types of cancer, including hematological malignancies, many types of carcinoma and soft tissue sarcomas [83]. Similar to PTX, DOX has been extensively reported and studied using PAMAM and other dendrimers. However, because of its water solubility, loading of DOX in dendrimer has several advantage over other drugs. DOX also has its own fluorescence properties, making it a useful drug to use to study the potential of dendrimers for anticancer drug delivery. Papagiannaros *et al.* [84] prepared two kinds of liposome using different molar ratios of hexadecylphosphocholine (HePC), egg yolk phosphatidylcholine (EPC) and stearylamine (SA). The DOXPAMAM dendrimer (3:1 and 6:1 molar ratio, respectively) was incorporated in the prepared liposome at an efficiency of approximately 91–95%. *In vitro* release of DOX from the liposomes was slow, only reaching approximately 17% at pH 7.4 even after 24 hours at room temperature. Lai *et al.* [85] reported photochemical internalization for DOX–PAMAM conjugates as an anticancer therapy. DOX was conjugated with a PAMAM dendrimer via pH-sensitive and -insensitive linkers and then combined with different photochemical internalization (PCI) strategies. The 'light after' PCI treatment was efficient in releasing DOX from PAMAM-hyd-DOX conjugates, leading to the increased accumulation of DOX and increased cell death through synergistic effects. In 'light before' PCI treatments, antagonism was observed. Both PCI techniques failed to improve the cytotoxicity of PAMAM-amide-DOX. Zhu *et al.* [86] reported partially PEGylated PAMAM for the targeted delivery of DOX. Acid-sensitive *cis*-aconityl and acid-insensitive succinic linkages were introduced between DOX and the polymer to produce PEG-PAMAM-*cis*-aconityl-DOX (PPCD) and PEG-PAMAM-succinic-DOX conjugates (PPSD), respectively. PPCD showed increased cytotoxicity against murine B16 cells, but cellular uptake was lower with increasing degree of PEGylation. PPSD showed more DOX accumulation in tumors at the same PEGylation degree, whereas PPCD showed lower hemotoxicity. It was demonstrated that attachment between the drug and polymer led to enhanced circulation time.

Zhang *et al.* [87] reported increased targeting of DOX by conjugating with RGD-modified PEGylated PAMAM dendrimers via acid-sensitive *cis*-aconityl linkages (RGD-PPCD) followed by binding with integrin overexpressed on tumor cells; the authors observed the controlled release of DOX in weak acidic lysosomes. To further explore this systems as therapy for gliomas. DOX was conjugated with PAMAM by acid-sensitive succinic linkage and further modified to form RGD-PPCD; for comparative studies, DOX was conjugated with PEG-PAMAM by acid-insensitive linkage to produce PPSD. He *et al.* [88] reported a dual-targeting drug carrier (PAMAM-PEG-WGA-Tf) based on the PEGylated fourth generation (G4.0) PAMAM dendrimer with transferrin (Tf) and wheat germ agglutinin (WGA) on the periphery for the delivery and targeting of DOX against brain tumor. Accumulation of DOX was higher in the tumor site and led to complete breakage of avascular C6 gliomas. Chang *et al.* [89] reported novel water-soluble and pH-responsive nanocarriers for DOX delivery. The authors conjugated DOX to PAMAM, which was then attached to super paramagnetic iron oxide NPs (IONPs). mPEG-PAMAM G2.5 dendrimers were used to stabilize the IONPs. DOX was attached to IONPs with a hydrazone bond, which is an acid-cleavable bond. The diameter of the conjugate after loading was 13.0 nm. Ajmal *et al.* [90] reported a complex of cationic dendrimer-DOX. The authors used a sixth-generation cationic poly-L-lysine dendrimer (DM) (MW 8149 kDa) for conjugation. This cationic dendrimer achieved higher penetration compared with free DOX into prostate 3D multicellular tumor spheroids. Higher anticancer activity was also reported *in vivo* in a Calu-6 xenograft animal model. Kojima *et al.* [91] reported an effect of collagen gels on the conjugation of dendrimers and DOX. They prepared DOX-dendrimer prodrugs with different surfaces, including PEG and collagen peptides, and reported that dendrimer-DOX conjugation with collagen gels was more cytotoxic against highly invasive MDA-MB-231 cells than against MCF-7, suggesting that these prodrug-embedded collagen gels would be useful for DDSs-associated metastasis.

Similarly, Li *et al.* [92] reported a dual-targeting carrier (G4-DOX-PEG-Tf-TAM) for DOX and tamoxifen, a PAMAM G4 dendrimer comprising transferrin (Tf) on the exterior and tamoxifen in the interior of the dendrimer for avoiding adverse effects during circulation

Trastuzumab

Trastuzumab (TZ) is a humanized monoclonal antibody, approved by the FDA for the treatment of human epidermal growth factor receptor 2 (HER2)-overexpressing early-stage and metastatic breast cancer and HER2-overexpressing metastatic gastric cancer through targeting of the extracellular domain of HER2, a tyrosine kinase receptor. TZ is approved for the treatment of breast cancer and is recommended as both a single agent and in combination with standard chemotherapy. Over the past few years, TZ has also been used as a targeting ligand [93]. Miyano *et al.* [94] reported trastuzumab conjugated with a KG6E dendrimer, resulting in HER2-specific binding, high cellular internalization rates, and trafficking to lysosome. These observations indicate that TZ-conjugated anionic amino acid dendrimers are promising for the selective delivery of TZ to HER2-expressing tumors.

Miscellaneous

It is not possible to fully explain the dendrimer mediated anticancer drug delivery with few examples mentioned earlier. Several other anticancer drugs and/or bioactives have been reported in combination with different types of dendrimer, including such as camptothecin derivatives and curcumin. Morgan *et al.* [95] reported encapsulation of camptothecin derivatives such as 10-hydroxycamptothecin (10HCPT) and 7-butyl-10 amino-camptothecin (BACPT) in a biocompatible dendrimer comprising natural metabolites, glycerol and succinic acid. The authors reported a 16-fold increase in the cellular uptake and increased retention when dendrimers were used as carriers. Similarly, Kong *et al.* [96] reported the delivery of 10-HCPT using multifunctional PAMAM G4 dendrimers. In this study, multifunctional dendrimers were prepared by grafting long hydrophobic C_{12} alkyl chains to improve and/or enhance drug loading, PEG chains for biocompatibility, and short cyclic peptide (RGDfK) ligands for tumor targeting on the surface of the dendrimers. The authors found that drug loading as well as water solubility (600-fold) was higher

compared with free 10-HCPT and that the drug–dendrimer complex was highly stable in PBS. Potent anticancer activity was reported against 22RV1 cells overexpressing integrin $\alpha_v\beta_3$, but were less active against MCF-7 cells. In another study, Kono *et al.* [97] designed PEG-modified PAMAM dendrimers and conjugated them with adriamycin. They prepared a G4 PAMAM dendrimer with a Glu residue and PEG was attached via the amino group of the Glu residue; in addition, adriamycin was attached via the Glu residue and an amide bond, [PEG-GLU(ADR)-G4], or hydrazone bond. The authors demonstrated that such hydrazone linkage was more stable than amide bonds and led to higher toxicity and efficacy even for MDR cells.

Markatou *et al.* [98] reported molecular interactions between dimethoxy curcumine and a PAMAM dendrimer. PAMAM G3.5 and G4 were used for this study and it was found that the drug was in its enolic form, and its interaction with PAMAM G4 involving the rearrangement of the terminal ethylene amino groups of the latter in a manner that affected markedly the whole amide spectrum of the dendrimer.

Concluding remarks

The overall advantages offered by the nanopolymeric architecture of dendrimers have attracted tremendous research attention over the past few decades, resulting in their development for use not only drug delivery, but also in the diagnosis and management of disease. Their use as a diagnostic is particularly important given that, the earlier the disease is detected, particularly cancer, the greater the chance that it can be successfully treated. In addition to their diagnostic use, the versatility of dendrimers has been shown through their use as gene-transfecting agents for the delivery of DNA and RNA. Dendrimers are evolving as a gold standard for the transfection of siRNA and other genes and for use in *in vivo* gene-delivery studies; the use of dendrimers to deliver gene–drug combinations directly to the target site, as well as other multimodel delivery approaches, is particularly exciting [99]. In addition, use of dendrimers in the design of electrochemical detectors is likely to encourage research to develop advanced tools that work in combination with the diagnostic and therapeutic potential of these multifunctional nanoarchitectural moieties.

Acknowledgements

The authors are grateful to, and would like to acknowledge, the University Grants Commission (UGC) New Delhi, India and Science and Engineering Research Board (SERB), Department of Science and Technology (DST), New Delhi, India, for providing research funding to U.G.

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