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Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications

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Dendrimers are members of a versatile, fourth new class of polymer architecture (i.e. dendritic polymers after traditional linear, crosslinked and branched types) [1]. Typically, dendrimers are used as well-defined scaffolding or nanocontainers to conjugate, complex or encapsulate therapeutic drugs or imaging moieties. As a delivery vector, the dendrimer conjugate linker or spacer chemistry plays a crucial part in determining optimum drug delivery to disease sites by conserving active drug efficacy while influencing appropriate release patterns. This review focuses on several crucial issues related to those dendrimer features, namely the role of dendrimers as nanoscaffolding and nanocontainers, crucial principles that might be invoked for improving dendrimer cytotoxicity properties, understanding dendrimer cellular transport mechanisms and the exciting role of dendrimers as high-contrast MRI imaging agents. The review concludes with a brief survey of translational efforts from research and development phases to clinical trials that are actively emerging.

Introduction

Dendrimers are a class of well-defined nanostructured macromolecules that possess narrow mass or size polydispersity and tree-like architecture distinguished by exponential numbers of discrete dendritic branches radiating out from a common core. These unique structural attributes confer a spherical shape to dendrimers after several layers of branching (i.e. generations) owing to surface congestion [1]. The resulting multifunctional dendrimer surfaces are amenable to a wide range of chemical modifications, and the interior is characterized by the availability of a substantial amount of solvent-filled void space that might be suitable for host–guest chemistry. The precise nanoscale sizes, shapes, surface chemistries and architectures of these dendritic macromolecules distinguish these materials as model systems for understanding a wide range of crucial nano-

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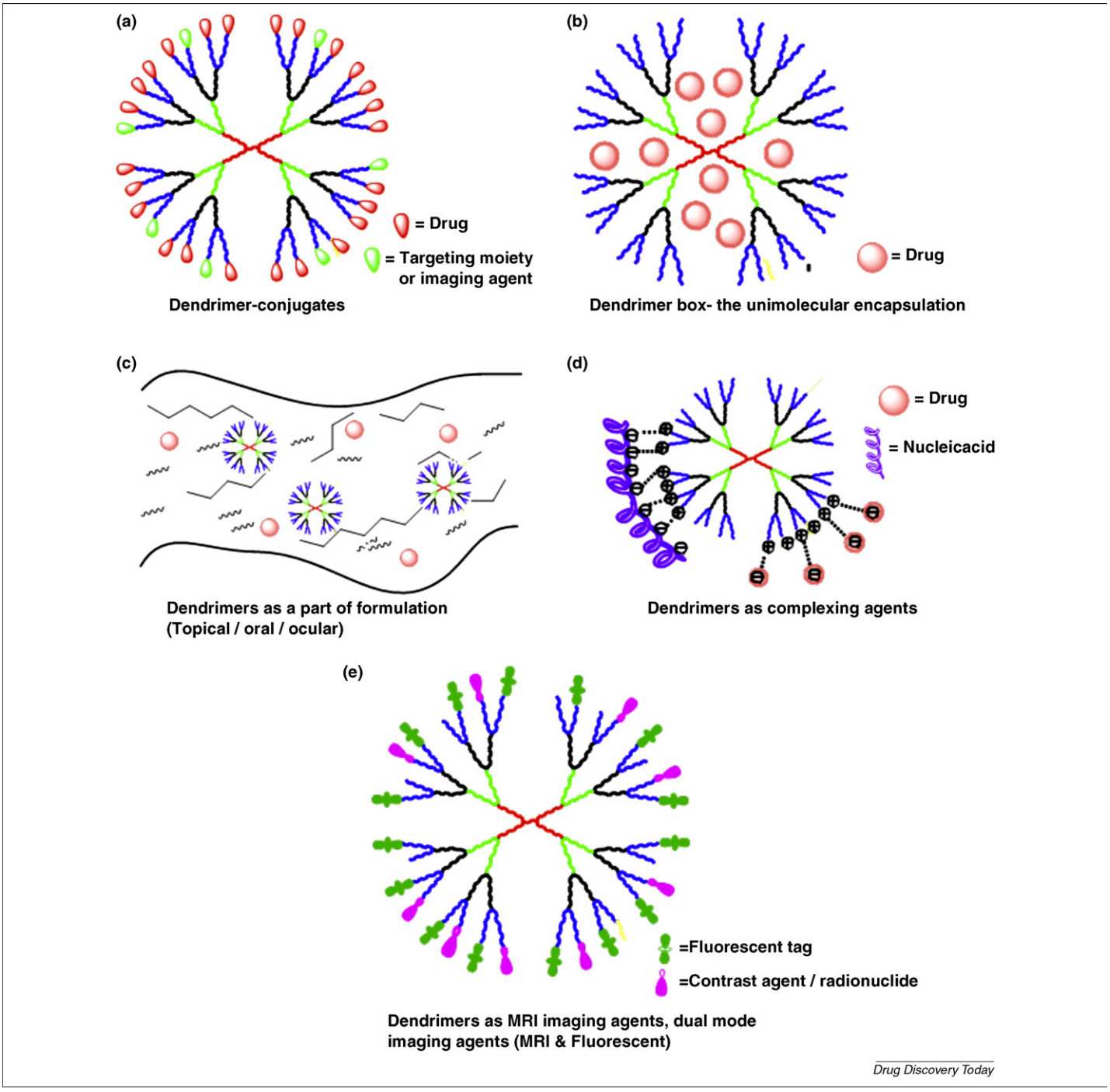

FIGURE 1

Potential applications of dendrimers. **(a)** Dendrimer drug conjugates, dendrimers linked to targeting moieties and imaging agents. **(b)** Encapsulation of the drugs in the dendritic interiors. **(c)** Dendrimers incorporated into various delivery systems for enhancing permeation, solubility and so on. **(d)** Dendrimers as complexing agents. **(e)** Dendrimers as carriers for MRI and fluorescent imaging.

medical issues, including polyvalent nanopharmaceuticals, DNA or siRNA delivery vectors, nanodiagnostics, MRI contrast agents and drug delivery, to mention a few (Fig. 1).

Wide varieties of compositionally differentiated dendrimers are available from several sources and historically annotated in Table 1. The Tomalia-type poly(amidoamine) (i.e. PAMAM, Starburst®) and Tomalia-type poly(etherhydroxylamine) (i.e. PEHAM, Priostar®) dendrimers, Meijer-type poly(propyleneimine) (i.e. PPI, Astramol®) dendrimers, Majoral-type phosphorous-containing dendrimers and Frechet-type poly(ether) dendrimers have all been exploited extensively for biomedical evaluation [1–5]. The first dendrimer family to be commercialized was the Tomalia-type PAMAM Starburst® series. This dendrimer family is the most referenced and well-characterized dendrimer class in the literature. These nanostructures are distinguished by their unique tree-like branching architecture that originates from an initiator core as concentric, symmetrical monomer

TABLE 1

Evolution of dendrimer synthesis strategies and valuable contributors

Method	Invented by or validated by	Year	Commercial status	Available from	Refs
Divergent synthesis	Tomalia *et al.*	1979–1984	STARBURST® PAMAM	National Dendrimer & Nanotechnology Center, Central Michigan Univ. Sigma Aldrich	[1–3,7–8][a]
	Newkome *et al.*	1985	Arborols	Frontier Scientific	[70]
	Meijer *et al.*	1993	ASTRAMOL® PPI	DSM Fine Chemicals, Sigma Aldrich	[71]
	Majoral *et al.*	1994			[72]
	Hult *et al.*	1993	Bolthorn®	Perstorp AB	[b]
	Simanek *et al.*	2006		Frontier Scientific	[73]
	Tomalia *et al.*	2005	Priostar® PEHAM/PEA	Dendritic Nanotechnologies	[74]
Convergent synthesis	Frechet *et al.*	1989			[75]
Self-assembling synthesis	Zimmerman *et al.*	1996			[76]
Lego chemistry	Majoral *et al.*	2003			[77]
Click chemistry	Sharpless *et al.*	2004			[78]
	Hult *et al.*	2009			[79]
	Carlmark *et al.*	2009			[114]

[a] PAMAM Dendrimers, http://www.chm.cmich.edu//ndc.html.
[b] Bolthorn® Dendritic Polymers, http://www.perstorppolyols.com/Sites/Polyols/Home/Boltorn.aspx.

branching shells. Each branching shell is termed a generation (G). For the PAMAM series, the size (i.e. diameter) increases by approximately 1 nm per generation and ranges from 1.1 to 12.4 nm as they proliferate from generations 1 to 10 [3,6,7]. The large number of identical chemical repeat units (i.e. symmetrical branch cell monomers) in the interior confers nanocontainer properties at generation = 4 and greater [6], whereas mathematically defined, exponential numbers of terminal groups/generations produce unique 3D structural presentations of surface moieties [7,8]. This offers an extraordinary combination of guest–host and interfacial surface functional advantages for the delivery of drugs, gene and imaging agents and tissue-targeting applications.

TABLE 2

Dendrimers as MRI contrast agents

Contrast/imaging agent	Dendrimer	MR application	Biodistribution studies in	Commercial/ clinical trial status	Refs
GD-DO3A MRI	G6- PAMAM-Cystamine	Breast cancer	Nude mice		[80]
(1B4M-Gd) 256	G6- PAMAM	Breast cancer	Mice		[81]
G6-Cy(5.5)1.25(1B4M-Gd)145 (Dual modality: MRI and FI)	G6-PAMAM	Sentinel (mammary) lymph nodes	Mice		[82]
(1B4M-Gd)1024	G8 PAMAM	Sentinel (mammary) lymph nodes	Mice		[83]
(1B4M-Gd)256	G6-PAMAM	Sentinel (mammary) lymph nodes	Mice		[84]
^{99m}Tc	G5-G7 PMPA	Kidney /Bladder	Copenhagen rats		[51]
(1B4M-Gd)64	G4 PAMAM	Kidney	Nude mice		[85]
SH L 643A, Gadomer-17	PAMAM	Coronary artery disease	Humans	Phase I clinical trials (by Schering plough)	[68]
Gd(III)-1B4M-DTPA & Rhodamine green (Dual modality: MRI and FI)	G2-PAMAM-Cystamine	Ovarian tumors	Nude mice		[86]
Gd-DTPA	G1-5 Poly(propylene imine) Dendrimers				[87]
^{111}In and Cy5, Alex (660,680,700,750) (Dual Modality: radionuclide and 5NIR)	G6 PAMAM	Optical lymphatic imaging and sentinel lymph nodes	Athymic mice		[88]
Gd(III) and Alexa Fluor 594 Dual Modality (MRI and FI)	G3 PAMAM	Tumors	Athymic mice		[89]
Gd(III))-1B4M-DTPA	G4 PAMAM	Angiography	Mice		[90]
(1B4M-Gd)192	G6 PAMAM	Intratumoral vasculature	Nude mice		[91]
(1B4M-Gd)64 (1B4MGd)64	DAB-Am64 (G6) DAB-Am64 (G4)	Liver micrometastasis	Nude mice		[92]
(1B4MGd)64	G4 PAMAM	Liver micrometastasis	Nude mice		[93]
(1B4MGd)*x*	G3-6 PAMAM	Blood pool	Nude mice		[94]

TABLE 3

Potential areas of dendrimer application and its versatile functions

Application	Active agent/drug	Dendrimer	Role	Commercial/ clinical trial status	Refs
Intracellular delivery	Colchicine	Glycopeptide	Carrier		[95]
Gene delivery & transfection agents	DNA & Cucurbituril	PPI-DAB dendrimer	Carrier		[96]
	DNA	PAMAM	Complexation		[97]
	DNA	PAMAM-arginine	Complexation		[98]
	DNA	PAMAM-G6	Complexation	Marketed as Superfect® (by Qiagen)	[99]
	DNA	PAMAM	Binding	Nanojuice Transfection Kit (by Starpharma) PrioFect™	[64]
	DNA/SiRNA	Priostar-PAMAM	Complex		[64]
	Oligo-DNA	G4-PAMAM	Complex		[100]
Antibody conjugates	60bca and J591, J591	G5-PAMAM	Carrier		[101]
		G5-PAMAM	Carrier		[102]
	Monoclonal sheep antibody	PAMAM	Carrier	Stratus® CS Acute Care™ NT-proBNP (pBNP) (Siemens Healthcare)	[103]
	Antibody	PAMAM	Binding (Anthrax detection)	Alert Ticket™ (by US Army)	[104]
Complexes	Mefenamic acid, Diclofenac, Amino salicylic acid	ClOC-PEG-COC G1-G3	Complexation		[105]
	Furosemide	G0-3 PAMAM			[61]
	Lamivudine	G5 propyleneimine (mannosylate)			[30]
	Indomethacin	G4PAMAM (folate surfacized)			[31]
Encapsulating agents	Etoposide	Core PAMAM –caprolactone-polyethylene glycol	Micelles		[106]
	Methotrexate	PAMAM	Liposomes		[107]
	Indomethacin	PEG-mesylate	Micelles		[108]
Transdermal	Indomethacin	G4-PAMAM (OH & NH_2)	Permeation enhancers		[57]
Ocular	Pilocarpine & Tropicamide	G1.5-4 PAMAM	Vehicle		[109]
	G3.5 PAMAM-glucosamine	G3.5 PAMAM	Therapeutic agent (Scar tissue inhibition)		[54]
	Porphyrin	Aryl ether dendrimer	Carrier (neovasculature)		[56]
Oral	Propranolol	G3 PAMAM	Solubility enhancer		[59]
	Naproxen	G0 PAMAM	Permeation enhancers		[17]
	Niclosamide	G0-3PAMAM	Solubility enhancer		[62]
	Sulfamethoxazole	G3 PAMAM	Solubility enhancer		[63]
	Furosemide	G0-3 PAMAM	Solubility enhancer		[61]
	Ketoprofen	G5PAMAM	Solubility enhancer		[110]
	Piroxicam	G3-4PAMAM	Complex/ Solubility enhancer		[111]
Parenteral	N-Acetyl cysteine	G4 PAMAM	Carrier		[12]
	Chloroquine Phosphate	G3-4 p-lysine-PEG(1000)	Encapsulating agent		[112]
Colon delivery	5 Amino salicylic acid	G3 PAMAM	Carrier	–	[20]
Topical gels	SPL7013	G4 lysine-based dendrimer	Therapeutic agent (anti-HIV agent)	VivaGel™ Phase II clinical trials (Starpharma)	[a]
	Nipedipine	G5 PAMAM	Solubility & permeation enhancers		[113]
	5-Fluorouracil	G2-G6 PAMAM	Permeation enhancer		[58]
***In vivo* injectables**	Amine, hydroxyl surface	G4 PAMAM	Therapeutic agent (anti-inflammatory)		[37]

[a] Please search for 'SPL7013 and HIV Infections' at http://www.clinicaltrials.gov.

The interesting nanoscale architecture of dendrimers confers several structural benefits over linear polymers, larger nanoparticles and liposomes. Such advantages include rapid cellular entry, reduced macrophage uptake, targetability and more facile passage across biological barriers by transcytosis. In comparison to linear polymers, dendrimers are multivalent owing to the presence of high multiplicities of reactive surface end groups, making them ideal drug carriers with higher drug payload capacities [9]. Encapsulation of drugs in PEGylated dendrimers can lead to enhanced permeation and retention (EPR) of the drug. The guest–host encapsulation properties of PAMAM dendrimers, based on its so-called 'unimolecular micelle or

dendritic box'-type architecture, has been demonstrated and often referred to as 'unimolecular encapsulation' [3,7]. Literature reveals extensive efforts directed toward the use of dendrimers as probes for MRI and fluorescent imaging (Table 2). The use of these versatile nanocarriers for the delivery of therapeutic agents to intracellular target sites for pain management, inflammation and oral, topical, ocular and transdermal delivery has also been explored (Table 3; Fig. 1). Several dendrimer-based diagnostic and/or *in vitro* technologies are already in the market (i.e. Stratus®, Siemens, Germany; Superfect®, Qiagen, Germany; and Priofect®/NanoJuice®, EMD-Merck, Germany); others are on the brink of commercialization, and several are in human clinical trials.

Dendrimer–drug conjugates: role of linking chemistry and its implications for drug release and efficacy

In recent years, there has been an explosion in the use of dendrimer-based nanocarriers for drug delivery. Conventionally, drugs are attached directly via linkers or spacers to dendrimer terminal groups and, in some instances, in combination with targeting moieties. Usually, ester or amide bonds are employed, which can be hydrolyzed inside the cell by endosomal or lysosomal enzymes [10,11]. A key feature for achieving improved drug delivery and efficacy is the ability to tailor the drug release from the dendrimer conjugate in an active form, at or in very close proximity to the target site and with minimum exposure to healthy collateral tissue. This might be accomplished by optimized biodistribution properties, passive targeting or receptor-mediated active targeting. Even though there has been substantial success in attaching multiple copies (i.e. therapeutic amounts) of drugs on various dendrimers, optimal release of drugs has been a challenge, not only with dendrimers but also with traditional polymer conjugates in general.

Recently, an interesting approach to overcoming the above limitations in clinically relevant drugs was exploited for intracellular and site-specific delivery of therapy. This strategy involved *N*-acetyl cysteine (NAC), which 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 (Fig. 2). NAC has two possible sites for conjugation: sulfhydryl function or carboxylic terminations. Esterification of NAC involving carboxylic groups was not explored; however, NAC was linked via the sulfhydryl groups. A key feature of this construct, involving NAC-type sulfhydryl moieties, is that they avoid plasma protein binding and thereby enhance bioavailability. This subtle design consideration enabled rapid *in vivo* NAC release in its active form using disulfide exchange reactions with indigenous intracellular glutathione. With this strategy, efficacy studies using microglial cells revealed that NAC–PAMAM conjugates were 16 times more efficacious than

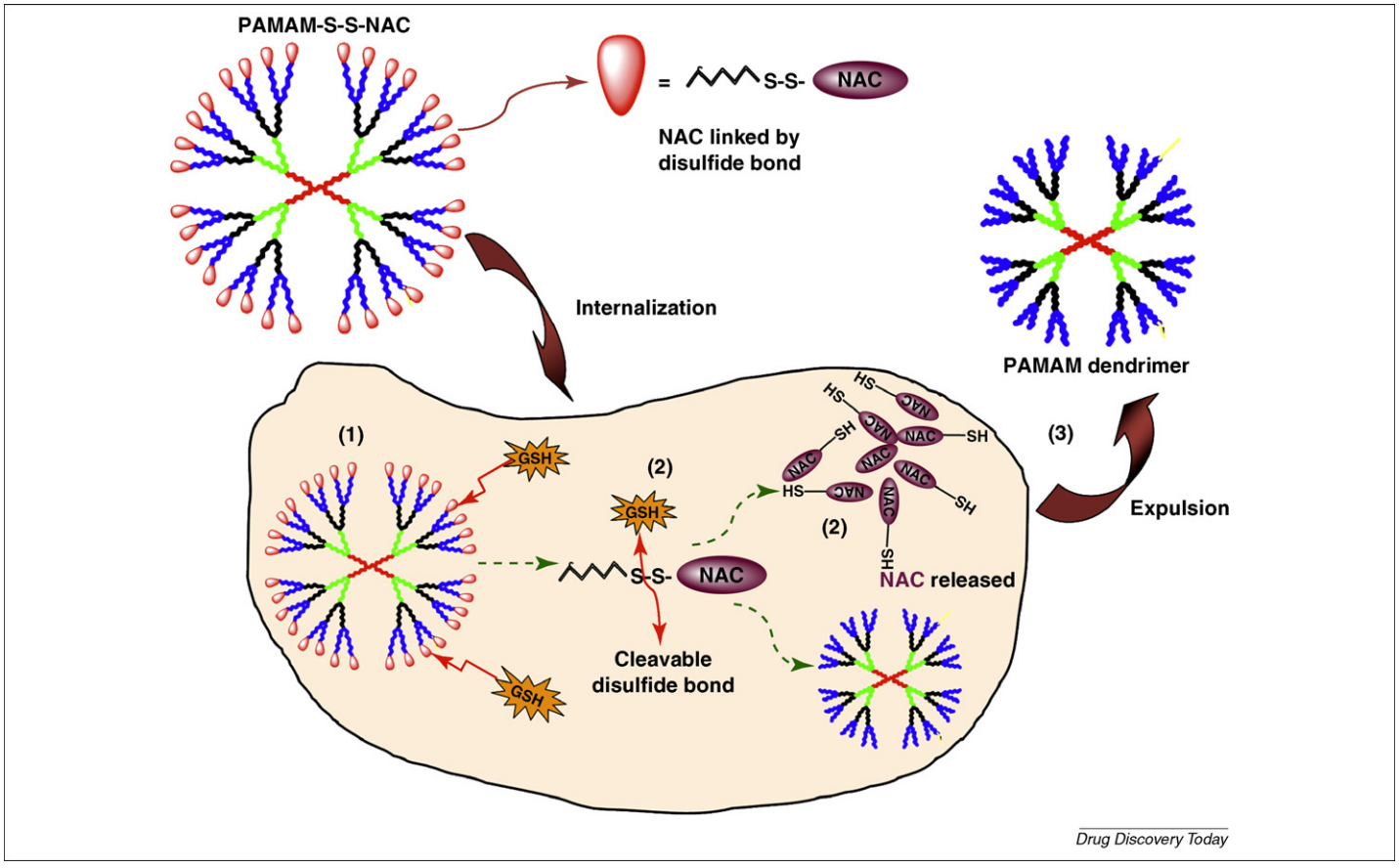

FIGURE 2

Schematic representation of the G = 4; PAMAM dendrimer linked to *N*-acetylcysteine by disulfide bonds. The dendrimer delivers the drug intracellularly by the cleavage of the disulfide linkage owing to the thiol exchange redox reactions initiated by the intracellular glutathione. The dendrimer carrier is then excreted by the cell.

the drug alone for the treatment of maternal fetal infections [12–14]. Interestingly, the release profiles from these NAC–PAMAM dendrimer conjugates were comparable to NAC release from PEGylated conjugates [15]. This study clearly demonstrates that the selection of an appropriate site for drug conjugation is of paramount importance to ensure optimal efficacy and release properties.

The fate of drug release from a conjugate is governed largely by the nature of the linking bond or spacer between the drug and scaffold and the targeted physiological domain for intended release. Ester and amide bonds might be cleavable by enzymes or under hydrolytic conditions; however, ester cleavage is generally more facile than amide cleavage for releasing drugs. Conjugates of Naproxen with G = 0; PAMAM dendrimers using both ester and amide linkages were studied extensively by Najlah *et al.* [16–17] to determine stability and release properties. The amide-linked conjugate resisted release in 80% human plasma, whereas the ester-linked conjugates underwent rapid esterase-catalyzed hydrolysis ($t_{1/2}$ = 51 min). The amide-linked conjugates exhibited stability in plasma and liver homogenate, whereas the ester-linked conjugates released the drug readily [17]. Quinidine attached to the anionic G = 2.5 and cationic G = 3; PAMAM dendrimers via ester bond using a glycine spacer, was released completely within 24 hours [9]. Venlafaxine linked directly to the G = 2.5; anionic PAMAM dendrimers via a hydrolyzable ester linkage was released in a sustained manner; with 50% being released within 18 hours [18]. Adriamycin (ADR) was conjugated to PEG-grafted G = 4; PAMAM dendrimers by amide and hydrazone linkage revealed that remarkable amounts of ADR (at endosomal pH 5.5) were released from the conjugates possessing hydrazone linkage compared with the amide linkage. Furthermore, those conjugates bearing hydrazone linkages exhibited seven times more efficacy than those with amide linkages [11]. Consistent with other studies, a recent study showed higher release profiles of ibuprofen linked through ester bond to G = 4; PAMAM dendrimers over the ibuprofen linked by amide bond [19].

It is well known that prodrugs containing azo bonds will release drugs owing to the presence of enzymatic azoreductase in the colon. Wiwattanapatapee *et al.* [20] selected *p*-aminobenzoic acid (PABA) and *p*-aminohippuric acid (PAH) as linkers to conjugate salicylic acid (SA) to dendrimers for colon delivery. PABA and PAH contain carboxylic groups that can form amide linkages with the amine surface groups of G = 3; PAMAM dendrimers. Furthermore, their aromatic amine groups can be used to facilitate diazotization and formation of azo bonds for linking to SA. The conjugates effectively released 45% and 57% of 5-aminosalicylic acid (active metabolite) from PAMAM–PABA–SA and PAMAM–PAH–SA, respectively, in the colon environment [20]. A recent study showed that the presence and absence of linker is found to immensely affect the drug release from PAMAM dendrimers. Ibuprofen conjugated by amide linkage to Gly-Phe-Leu-Gly peptide linker appended to G = 4 PAMAM–NH_2 dendrimer was found to release 40 times more in cathepsin B solution in 48 hours than Ibuprofen conjugated by amide linkage to Gly-Phe-Leu-Gly peptide linker appended to G = 4 PAMAM–NH_2 dendrimer was found to release 40 times more in cathepsin B solution in 48 hours than the ibuprofen linked directly by amide linkage to same dendrimer [19]. Yet another recent study showed the impact of linker choice on Doxorubicin (Dox) release from dendrimers. Dox conjugated by acid-sensitive *cis*-aconityl linkage was found to release at lysosomal pH 4.5 and at pH 7.4, as compared to the acid-insensitive succinic linkage [21]. The ALA (5-aminolaevulinic acid) linked to the dendrimer by ester linkage was released, leading to the synthesis and accumulation of porphyrins in thicker tumors [22]. Clearly, the appropriate choice of linkers in concert with anticipated physiological compartment chemistry is crucial for designing effective drug release properties for dendrimer conjugates.

Dendrimer–drug linking chemistry not only influences drug release properties but also has a profound impact on efficacy. For example, free methotrexate (MTX) was released upon internalization of MTX–dendrimer conjugates owing to the hydrolysis of ester bond in an acidic endosomal environment [23]. MTX has two possible sites for conjugation owing to the presence of carboxylic and amine terminations. MTX linked to acetamide-functionalized G = 5; PAMAM dendrimer via ester bond was found to be four times more active than free MTX, whereas MTX linked by amide bonds to the same dendrimer was less active than free MTX [23,24]. Interestingly, dendrimer surface functionality might play a crucial part in the efficacy of certain conjugates. For example, when MTX was linked with amide bonds involving the carboxylic acid groups of the anionic G = 4; PAMAM dendrimers, these conjugates were found to be 24 times more active than free MTX, even in MTX-resistant cancer cells. When MTX was linked by amide bond to amine-terminated G = 4; PAMAM dendrimers involving the carboxylic acid groups on MTX; however, the conjugate was found to be considerably less active than free MTX [25]. Similar findings were reported for PAMAM dendrimer–cisplatin conjugates formed by using amide bonds, which showed 100 times less cytotoxicity against human lymphoblastoid leukemia cell line CCRF-CEM [2].

In the past, there have been reports describing considerable loss in enzymatic activity (67% and 50%) upon conjugation of streptokinase (SK) to PEG and dextran, respectively, wherein the conjugates were less efficacious than free SK. Interestingly, the efficacy of various SK–PAMAM; G = 3.5 conjugates were evaluated, and it was found that a 1:1 ratio retained highest enzymatic activity (80%). The SK–dendrimer conjugates exhibited rapid *in vitro* clot lysis comparable to that of free SK, and these constructs are expected to have much better stability and longer circulation times than free SK [26]. Retention of efficacy after conjugation is crucial and proper choice of scaffolds can overcome such loss in activity.

The conventional strategy for releasing covalently bound dendrimer–drug conjugates is to initiate a pH-dependent chemical trigger to release a drug molecule from each terminal conjugation site of the dendrimer. A clever and novel concept that enables simultaneous release of all dendrimer functional groups by a single chemical trigger has been reported by de Groot, Shabat and McGrath [27,28]. Various terms have been coined to describe this release mechanism, such as 'cascade-release dendrimers', 'dendrimer disassembly' and – colorfully – 'self-immolative dendrimers'. De Groot and colleagues [29] demonstrated the release of paclitaxel (Taxol) by this mechanism and found that the dendrimer degradation products were not cytotoxic – except for paclitaxel itself. However, more evaluation is required to determine the

viability of this concept, based on concerns that drug release at the wrong time and place could lead to devastating results [28].

The release of physically entrapped guest-type drugs in dendrimeric hosts might be controlled by both surface modification and the density of modifying groups. For example, surface modification of PPI dendrimers; (G = 4)[a] with mannose resulted in Lamivudine encapsulation conjugates, which exhibited slower release rates (i.e. pH 7.4 PB buffer). These observations were attributed to increased steric hindrance and the presence of a large number of functional groups available for complexation [30]. Similar observations were reported by Chandrasekar *et al.* [31], where indomethacin loading was enhanced by increasing the multiplicity of folate groups on the surface of G = 4; PAMAM dendrimers. A slow release of indomethacin was observed from these dendrimers owing to the sterically encumbering large folate groups. Finally, conjugation of tuftsin on the surface of G = 4; PPI dendrimers (see footnote a) resulted in enhanced entrapment of efavirenz and sustained its release up to 144 hours [32].

Even as the studies investigating the drug release patterns from dendrimers based on different linking chemistry continue, the newer hypothetical approaches based on molecular modeling to predict the drug release based on breaking point on an enzymatic attack are being explored [33]. These new studies provide an insightful evaluation approach to direct the synthesis of the polymer drug conjugates and predict their behavior in the biological environment. In conclusion, it is important to consider dendrimer–conjugate linking chemistry, as well as appropriate use of spacers and drug structure–activity relationships, in the design of optimum dendrimer–drug conjugates. In future efforts to optimize drug or active agent release profiles for dendrimers it will be crucial to understand all safety and biodistribution issues related not only to the dendrimer conjugates but also to the independent drug and naked dendrimer vectors.

Dendrimers/dendrimer conjugates: strategies for modulating and minimizing cytotoxicity

Dendrimers have shown enormous potential as nanocarrier/delivery systems because they can cross cell barriers by both paracellular and transcellular pathways. As is the case for most cationic macromolecular systems, one must pay particular attention to cationic dendrimer-based delivery systems, especially when large doses are required. Cytotoxicity and permeability profiles for most PAMAM dendrimers are found to be concentration, generation and surface charge dependent. Unequivocally, both anionic and neutral PAMAM dendrimers were found to be substantially less cytotoxic and exhibit lower permeation rates than cationic dendrimers. Lower generation (i.e. G = 0–1) dendrimers tend to exhibit considerably less cytotoxicity and permeation than higher generation (i.e. G = 2–4) dendrimers [5,17,34].

Several effective strategies have emerged for modulating or minimizing dendrimer-related cytotoxicity issues. These strategies have generally involved unique surface modifications, optimized nanosize control (i.e. <8 nm) for rapid renal clearance, or creative enhancements of delivery efficacy related to more effective passive or active targeting. Several approaches have been explored and include enhanced targeting efficiency to minimize collateral exposure to healthy tissue, increased permeability for enhanced absorption, increased gene transfection efficiency and suppression of charge-related (i.e. cationic) cytotoxicity by cloaking surface charge with appropriate dendrimer surface modifications. Passive targeting efficiency is increased dramatically by choosing appropriate nanoscale dendrimer sizes for maximizing EPR effects or organ-specific localization, as demonstrated by the use of certain dendrimer-based MRI imaging agents [35].

Alternatively, receptor-mediated active targeting might be optimized by appropriate design of dendrimer conjugates. This includes conjugating optimum multiplicities of targeting moieties to dendrimer surfaces with appropriate spacers that will allow appropriate receptor-mediated recognition space for maximum avidity to targeted disease sites. A wide range of small-molecule ligands (vitamins, cell-penetrating peptides, antibodies and antibody fragments and so on) have been demonstrated against tumor-associated antigens. For example, G = 3.5; PAMAM dendrimers possessing PEG as a spacer linked to folic acid showed improved targeting of indomethacin to inflammatory tissues [31]. Surprisingly, certain naked PAMAM dendrimers (i.e. amine and hydroxyl moieties) have recently been noted to exhibit intrinsic anti-inflammatory activity comparable to indomethacin [36]. Biotinylated G = 5; PAMAM dendrimers, however, have shown increased uptake in HeLa cells (cervical cancer), wherein uptake was specific to the cancer cells [37].

Work reported by Baker *et al.* [38] has shown that cytotoxicity related to targeted amine-terminated PAMAM dendrimer conjugates for cancer therapy could be minimized by surface amidation of amine moieties. In another case, amidation of dendrimer amine surface groups led to a tenfold reduction in cytotoxicity while retaining desirable transepithelial permeability across Caco-2 cell monolayers [39]. Partial amidation was effective in reducing the cytotoxicity, whereas complete amidation showed the absence of cytotoxicity. A linear relationship between the number of naked amine surface groups and cytotoxicity was observed; however, it was interesting to note that these surface-modified PAMAM dendrimers displayed permeability and uptake profiles similar to those of unmodified (native) dendrimers [39]. In another study, it was shown that shielding (i.e. cloaking) the dendrimer with four chains of PEG 2000 and six chains of lauroyl functionality led to reduced toxicity of cationic PAMAM (G = 2–4) dendrimers toward Caco-2 cells, although the unmodified dendrimers were appreciably cytotoxic compared with the surface-modified dendrimers. Clearly, it has been well demonstrated that suitable

[a] Please note this generational nomenclature has been revised from the incorrectly reported G = 5; PPI dendrimer nomenclature to make it consistent with accepted literature nomenclature. Many PPI examples have been documented in the literature based on incorrect generation nomenclature initiated by DSM during their commercial introduction of PPIs in the 1990s. This nomenclature has been inconsistent with the literature, as well as with the mathematics used to predict the number of surface groups as a function of generation. (i.e. $Z = N_c N_b^G$, where Z equals the number of dendrimer terminal groups). A correction was made as early as 1999 by DSM employee Margaret Rookmaker in Ref. [71] and again, more recently, in Ref. [34]. This correction is often overlooked and leads to confusion when making generational comparisons between Frechet-type poly(ether) dendrimers, Tomalia-type PAMAM dendrimers and other dendrimer systems. The conversion necessary for transforming from incorrect DSM nomenclature to the correct literature designations is G(literature) = G(DSM) − 1. This correction is especially important when comparing the number of surface groups in PAMAM dendrimers with the PPI dendrimer series.

shielding of cationic charge on a dendrimer surface by attached chains can substantially suppress cytotoxicity [40].

It has been shown that PEGylation of PAMAM dendrimers yields stealth-type dendrimers with high biocompatibility. This is demonstrated by a 40% increase in endothelial cell viability after 24 hours incubation with PEG–PAMAM dendrimers compared with native PAMAM dendrimers [41]. A recent study by Jacobson *et al.* [42] compared G = 3, amine-terminated PAMAM dendrimers conjugated with PEG groups of various sizes (Mn = 550, 750 and 2000) followed by *N*-acetylation. This study showed that the cytotoxicity of these PEG–dendrimer conjugates (PEG Mn = 550, 750) was twofold to ninefold lower than *N*-acetylated dendrimers (i.e. 14 acetyl groups) but containing no PEGylation [42].

Intracellular trafficking: influence of dendrimer generation (size) and surface charge

Several mechanisms have been proposed to describe molecular transport across cell membranes (i.e. endocytosis, passive diffusion and carrier-mediated and paracellular transport). Depending on surface properties (i.e. charge and size), PAMAM dendrimers are generally more effectively transported across epithelial barriers than many conventional linear water-soluble polymers [39,43]. The epithelial permeability of cationic dendrimers decreases with size, whereas the permeability of anionic dendrimers increases with their size. Charged dendrimers, however, exhibit greater permeability than neutral dendrimers (i.e. G = 2; –OH terminal), which have no net surface charge at physiological pH to perturb or disrupt anionically charged cell membranes. It goes without saying that a comparison of cationic and anionic dendrimers shows that cationic dendrimers exhibit higher permeability [35]; and the cationic dendrimer transport is assisted by a combination of paracellular and endocytic mechanisms.

Recent studies have revealed that apart from the three important parameters surface charge, molecular weight and generation, PAMAM dendrimer internalization or endocytosis properties might be largely dependent on the targeted cell type. For example, Kitchens *et al.* [44] reported that the trafficking of tritium-labeled, cationic G = 4; PAMAM dendrimers to endosomal and lysosomal compartments in Caco-2 cells was found to be rapid and mediated by a clathrin-dependent endocytosis mechanism. This mechanism was further confirmed based on lowered uptake and permeability of G = 4; PAMAM–NH_2 in the presence of endocytosis inhibitors such as brefeldin A, colchicine, filipin and sucrose [44]. In another study by Perumal *et al.* [45], it was shown that anionic G = 4; PAMAM dendrimers are internalized by caveolae-mediated endocytosis in A549 lung epithelial cells, whereas cationic and neutral G = 4; PAMAM dendrimers are internalized by a nonclathrin, noncaveolae-mediated mechanism involving electrostatic interactions or other nonspecific fluid-phase endocytosis. These studies demonstrated that cationic dendrimers are found in peripheral vesicles, unlike the anionic and neutral dendrimers, which are generally found in the lysosomes [45].

In a study by Saovapakhiran *et al.* [46] intracellular trafficking of amine-terminated G = 3; PAMAM dendrimers was studied in a human colon adenocarcinoma HT-29 cell line. The naked dendrimer and dendrimer–propranolol conjugate (i.e. two propranolol molecules) exhibited caveolae-dependent endocytosis and macropinocytosis pathways, whereas a lauroyl-functionalized-dendrimer–propranolol conjugate (i.e. two molecules each of lauroyl and propranolol) exhibited internalization by caveolae-dependent, and possibly clathrin-dependent, endocytosis pathways. The simple dendrimer–lauroyl conjugate (i.e. two lauroyl molecules) internalized via caveolae-dependent, clathrin-dependent and macropinocytosis pathways. Parallel subcellular colocalization results showed that unmodified and all surface-modified G = 3; PAMAM dendrimers were internalized and trafficked to endosomes and lysosomes [46]. These recent findings provide insights that enable one to speculate on the implications of intracellular conjugate localization for drug release and efficacy. Correlating these results with the efficacy of various dendrimer–MTX conjugates helps to explain why the amide-bonded MTX–anionic dendrimer conjugates were 25 times more efficacious than the amide-bonded MTX–cationic dendrimer conjugates. The higher localization and residence time of the former in lysosomes might have contributed to this observed effect [45].

To demonstrate the influence of size and charge on transport properties of PAMAM dendrimers across Caco-2 cells, Kitchens *et al.* [47] carried out extensive studies on cationic, neutral and anionic dendrimers. It was found that permeability was enhanced with an increase in the number of anionic surface groups in the PAMAM–COOH series. However, cationic, amine-terminated G = 2; PAMAMs exhibited greater permeability than neutral, hydroxyl functionalized G = 2; PAMAMs and anionic, carboxylated G = 1.5 or anionic, carboxylated G = 2.5; PAMAM dendrimers. On the contrary, anionic, carboxylated G = 3.5; PAMAM dendrimers exhibited greater permeability than the smaller dendrimers G = 1.5–2.5-COOH, G = 2-NH_2 and G = 2-OH without reducing cell viability. Anionic dendrimer G = 3.5-COOH and cationic G = 4-NH_2 modified with eight fluorescein isothiocyanate (FITC) moieties showed the highest transport rates; attachment of the hydrophobic FITC label seemed to increase permeability while reducing toxicity of the amine-terminated G = 4; PAMAM conjugates [47].

Kitchens et *al.* [47] showed that amine-terminated PAMAM–NH_2 dendrimers decreased transepithelial electrical resistance (TEER) readings and increased ^{14}C-mannitol permeability as a function of generation number and dendrimer size. Interestingly, neutral PAMAM–OH dendrimers did not influence TEER or ^{14}C-mannitol transport, whereas anionic, PAMAM–COOH dendrimers decreased TEER and increased ^{14}C-mannitol permeability within a specific size window (i.e. G = 2.5–3.5). These investigators ranked the order of PAMAM dendrimer permeability as G = 3.5-COOH > G = 2-NH_2 > G = 2.5-COOH > G = 1.5-COOH > G = 2-OH. The enhanced PAMAM permeability was attributed to opening of tight junctions in the cells. It seems that by appropriate engineering of the PAMAM dendrimer surface chemistry, it is possible to increase polymer transepithelial transport, which is beneficial for the oral delivery of poorly soluble drugs [47]. Finally, polyamines: ornithine- and arginine-conjugated PAMAM dendrimers also showed enhanced permeability and reduced TEER readings, thus indicating paracellular transport across the Caco-2 cells [48].

Dendrimers as intracellular drug and gene delivery agents

Appropriate surface-functionalized dendrimers can enter certain cells remarkably well and, hence, are under active investigation as

potential drug delivery and gene-transfection agents (Table 2). The objective is to deliver a therapeutic drug or gene payload to a specific intracellular site for desired local action. The intracellular delivery of dendrimer nanocarriers involves both extracellular drug release at the interstitium (tissue site) and intracellular delivery upon internalization. It is essential that the dendrimer nanocarrier loaded with a drug or gene is not cleared too quickly from circulation. The design of a suitable delivery system requires elimination or minimization of all nonspecific interactions that might occur between the dendrimer vector and the environment of the systemic compartment. A primary function of the carrier is to mask all unwanted interactions between the drug and the environment until the drug is released from the carrier at the target site.

Intuitively, the effective release of therapeutic drugs from dendrimer conjugates after internalization into a disease site might be considered a crucial event for efficacy. Interestingly, this is not essential in all instances and some dendrimer conjugates exhibited efficacy in their conjugated forms. One such system includes methylpredinisolone–PAMAM; G = 4-OH conjugates, which carry a payload of 12 drug molecules per dendrimer. These dendrimer conjugates have shown high cellular uptake in human lung carcinoma epithelial cell line, wherein the conjugate activity was found to be comparable to that of free drug [49]. It has been found that some drug–dendrimer conjugates are rapidly internalized into cells, where the conjugated drug can be released slowly over longer time periods. For example, ibuprofen complexed with PAMAM dendrimers bearing amine groups (i.e. G = 3–4) exhibited rapid uptake (i.e. >90% in one hour) in A549 cells. This dendrimer assisted internalization was substantially faster than that observed for the pure drug (which took longer than three hours). Upon internalization, therefore, the complex provides a very efficient and sustained delivery of ibuprofen [50]. It is interesting to note that covalently linking ibuprofen to hydroxyl-terminated PAMAM dendrimers produced a very high dendrimer–drug conjugate payload (i.e. 58 ibuprofen molecules per dendrimer). Furthermore, the conjugate was shown to exhibit an enhanced cellular uptake, with significant amounts (i.e. ~30%) being internalized in 15 min. Efficacy studies on the conjugate indicated rapid prostaglandin suppression within 15 min *vis-a-vis* the pure drug, which required 60 min to exhibit comparable activity to that of the conjugate [10].

Dendrimers: microvascular extravasation properties

An ideal polymeric carrier suitable for parenteral administration should be essentially nontoxic, nonimmunogenic and, if at all possible, biodegradable. The carrier should display suitable tissue distribution attributes that confine the therapy to the targeted disease site and, ideally, avoid exposure to collateral healthy cells and/or tissue. In the case of dendrimer–drug conjugates, the two most probable metabolites that must be considered include the dendrimer and the drug itself. As such, the fate of each metabolite needs to be documented. Hence, the toxicity, biodistribution and retention or excretion properties of the polymeric carrier are of paramount importance in the early screening of polymeric vectors.

Extravasation is the movement of smaller, lower molecular weight molecules from the blood circulatory system across the endothelial lining of capillary walls into the neighboring interstitial tissues. Efficacious drug delivery systems must extravasate from the systemic circulation across the microvascular endothelium into the interstitial tissue to reach a targeted site of therapeutic action. The influence of size and molecular weight within a series of PAMAM–NH_2 dendrimers on extravasation across the microvascular endothelium was investigated by Kitchens *et al.* [43]. Extravasation time(s) increased exponentially with an increase in molecular weight and size of the PAMAM dendrimers. The order of extravasation time for PAMAM–NH_2 dendrimers was G0 < G1 < G2 < G3 < G4, ranging from 143.9 to 422.7 s. This size-dependent selectivity is due to the increased exclusion of PAMAM–NH_2 dendrimers from the endothelial pores, 4–5 nm in radius, as the dendrimer size was increased. On the basis of the reported molecular sizes of PAMAM–NH_2 dendrimers, ranging 1.5–4.5 nm, it seems that dendrimers can cross the microvascular endothelium through the endothelial pores of diameter 4–5 nm if their dimensions are small enough. The observed extravasation of PAMAM–NH_2 dendrimers might be a function of the electrostatic interactions between the dendrimer and the negatively charged endothelium glycocalyx lining. The study demonstrated that an increase in molecular weight and size of polymers results in increased extravasation time across the microvascular endothelium. Molecular geometry and surface charge also influences the microvascular extravasation of water-soluble polymers across the endothelial barrier. As such, spherical, positively charged PAMAM dendrimers usually exhibit shorter extravasation times than linear macromolecules such as PEGs.

Biodistribution of ^{99m}Tc radiolabeled G = 5–7; poly (2,2-bis(hydroxymethyl) propanoic acid) (PMPA) dendrimers was determined noninvasively with rat models *in vivo*. This study showed that the path taken by these dendrimers from tail vein to bladder was very rapid and they were efficiently cleared from circulation via the kidney within 15 min of injection. On the basis of size, rapid clearance of this dendrimer series could be attributed to the lower molecular weights (i.e. 4.1, 7.8 and 15.2 kDa for G = 5, 6 and 7, respectively), which are well below the renal filtration cutoff of 40–60 kDa. This was corroborated with radioactivity measurements conducted on various harvested organs and tissues. Within 5 min of injection, 95% of the dose was eliminated, and 99% of dendrimer was cleared in 15 min [51].

EPR has been widely used to achieve passive tumor localization of macromolecular anticancer agents to angiogenic solid tumors. The intravenous injection of platinate conjugate with G=3.5 PAMAM dendrimer resulted in an approximately 50-fold increase in selectively targeting platinum reagents to subcutaneous B16F10 tumors as compared to intravenous administration of cisplatin at its maximum tolerated dose [2].

In the early 1990s, Lauterbur, Wiener and Tomalia *et al.* [52] pioneered the first use of PAMAM dendrimers as unique nanoscaffolding for the conjugation and presentation of high multiplicities of complexed gadolinium moieties (i.e. Magnevist® type moieties) [3]. These dendrimer-ligated gadolinium conjugates provided very high multiplicities of gadolinium on the surface of monodisperse nanospheroids and exhibited ideal rotational correlation coefficients. The combination of these two features produced some of the highest known relaxivity values (T_1) at that time. It was demonstrated that relaxivity values (T_1) increase

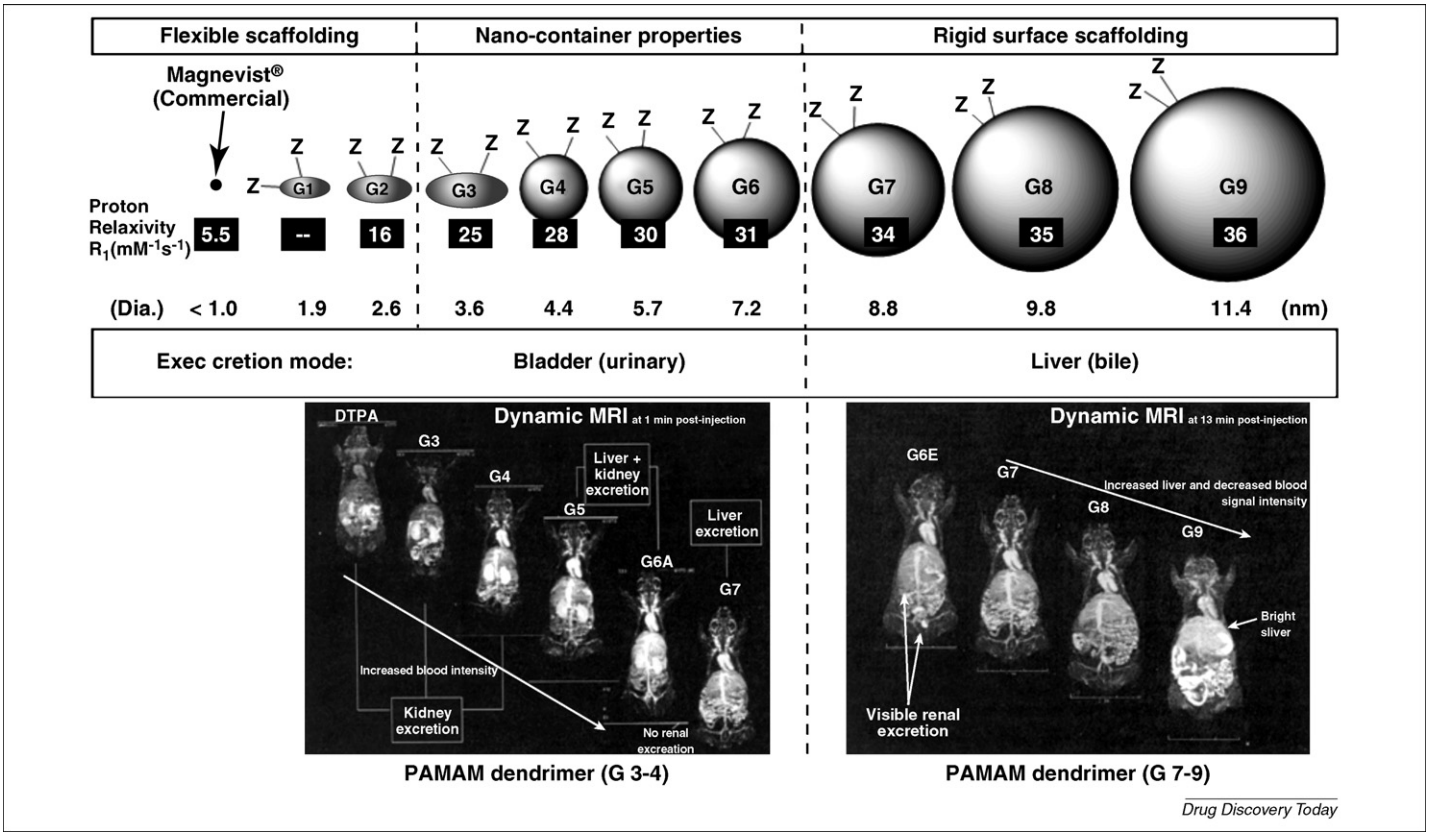

FIGURE 3

Dendrimer scaffolding dimensions for presenting MRI imaging. **(a)** Scaled spheroids illustrating the relative sizes (nm) for G = 1–8; Tomalia-type PAMAM dendrimer series, wherein the (core: 1,2-diaminoethane; G = 1–8); [dendri-PAMAM$(NH_2)_{NcNb}{}^{G}$] generational series is categorized into the observed periodic properties of (i) flexible scaffolding (G = 1–3), (ii) nanocontainer properties (G = 4–6) and (iii) rigid surface scaffolding (G = 7 and greater), owing to enhanced surface congestion as a function of generation. **(b)** MRI images of mice using Magnevist®-modified PAMAM dendrimers, namely (core: 1,2-diaminoethane; G = 1–8); [dendri-PAMAM$(NH_2)_{NcNb}{}^{G}$], wherein Magnevist and G = 3–4 are excreted completely through the kidney, G = 5 is excreted through both kidney and liver, and G = 6–9 are excreted exclusively through the liver. *Note*: G = 3–9 are excellent 'blood pool' agents relative to Magnevist (i.e. diethylenetriaminepenta-acetic acid), and G = 9 is organ specific for the liver, presumably because of its large nanosize [53]. MRI images courtesy of National Institutes of Health

as a function of dendrimer generation and are illustrated in Fig. 3.

Higher (T_1) values are a desirable property associated with optimum MRI imaging agents required for early stage disease detection. These early dendrimer-based MRI contrast agent investigations have been actively extended by major investigators, including Kobayshi and Brechbiel [53]. Their recent work has demonstrated that these unique dendrimer-based MRI agents exhibit size-dependent, organ-specific 'passive targeting' properties, as illustrated in Fig. 4.

On the basis of the well-defined nanoscale sizes associated with this generational continuum of PAMAM dendrimer-based MRI agents (i.e. G = 1–8), it has been shown that G = 4 and larger PAMAM contrast agents are large enough to preclude extravasation and function nicely as blood pool agents. Furthermore, this generational series of MRI agents has enabled them to use these size-calibrated agents for quantitating renal excretion properties as a function of nanoparticle size. Figure 3 clearly illustrates that this nanoparticle size cutoff occurs at G = 6–7 and corresponds to a nanoparticle size of approximately 8 nm. An overview of these crucial 'passive targeting' and 'renal excretion' properties as revealed by these PAMAM dendrimer-based MRI imaging agents is described in Fig. 5.

Other pharmaceutical and personal care applications

Dendrimers have been evaluated for many other applications apart from targeted and intracellular delivery agents. For example, concomitant administration of glucosamine and glucosamine 6-sulfate dendrimers increased the long-term success of glaucoma filtration surgery (from 30% to 80%) in rabbit models by dramatically reducing scar tissue formation [54]. In another study, anionic dendrimers were used to deliver an antivascular endothelial growth factor (oligonucleotide, or ODN-1) to inhibit laser-induced choroidal neovascularization (CNV) in the eyes of rat. The long-term delivery (four to six months) of ODN-1 from dendrimer did not elicit immunogenic or considerable inflammatory response and showed considerably greater inhibition of CNV than ODN-1 administered alone [55]. Dendrimer–porphyrin (DP) conjugates were used in a photodynamic therapy application to treat corneal neovasculature induced in mice. These DP conjugates did not traverse to the normal ocular vessels but selectively entered into the neovasculature, which limited the side-effects substantially. Both the DP conjugate and DP conjugate in micelles exhibited efficacy [56]. Glucosamine G = 3.5; PAMAM–COOH dendrimer conjugates exhibited immunomodulatory and antiangiogenic properties. These studies infuse the applicability of dendrimers as carriers in ocular delivery of drugs.

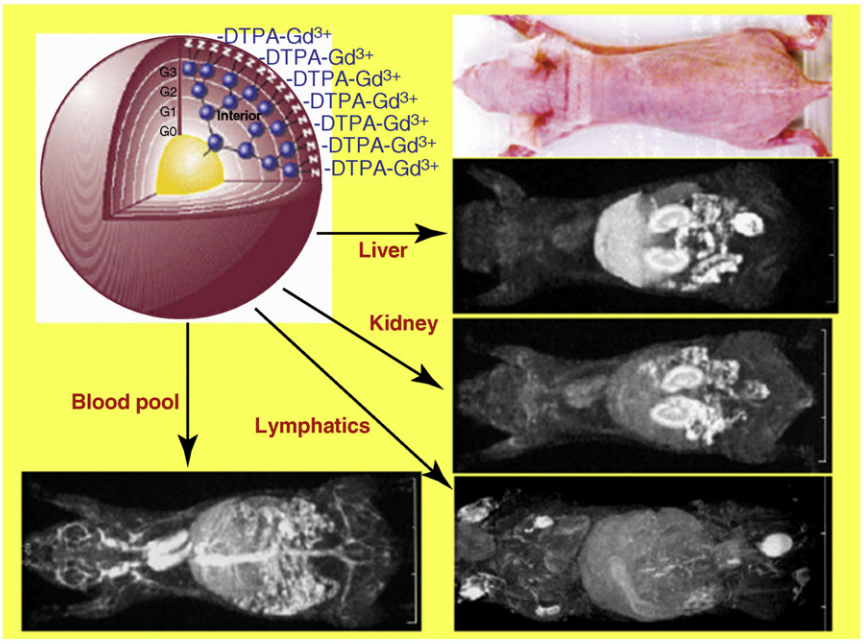

FIGURE 4

Nanosize-selective PAMAM-dendrimer-based MRI contrast agents as a function of dendrimer generation and organ specificity. Image courtesy of H. Kobayashi and M. Brechbiel, National Institutes of Health.

Dendrimers have been effective as transdermal and topical drug delivery systems for nonsteroidal anti-inflammatory drugs and antiviral, antimicrobial, anticancer or antihypertensive drugs. Dendrimers have been extensively evaluated as permeability enhancers in transdermal drug delivery for hydrophobic drugs. Bioavailability and transport of indomethacin through intact rat skin was enhanced *in vitro* and *in vivo* by using G = 4-PAMAM (OH and NH_2) dendrimers with *in vivo* steady-state flux being achieved in five hours [57]. Extensive evaluation of dendrimers as permeation enhancers for topical delivery of poorly soluble

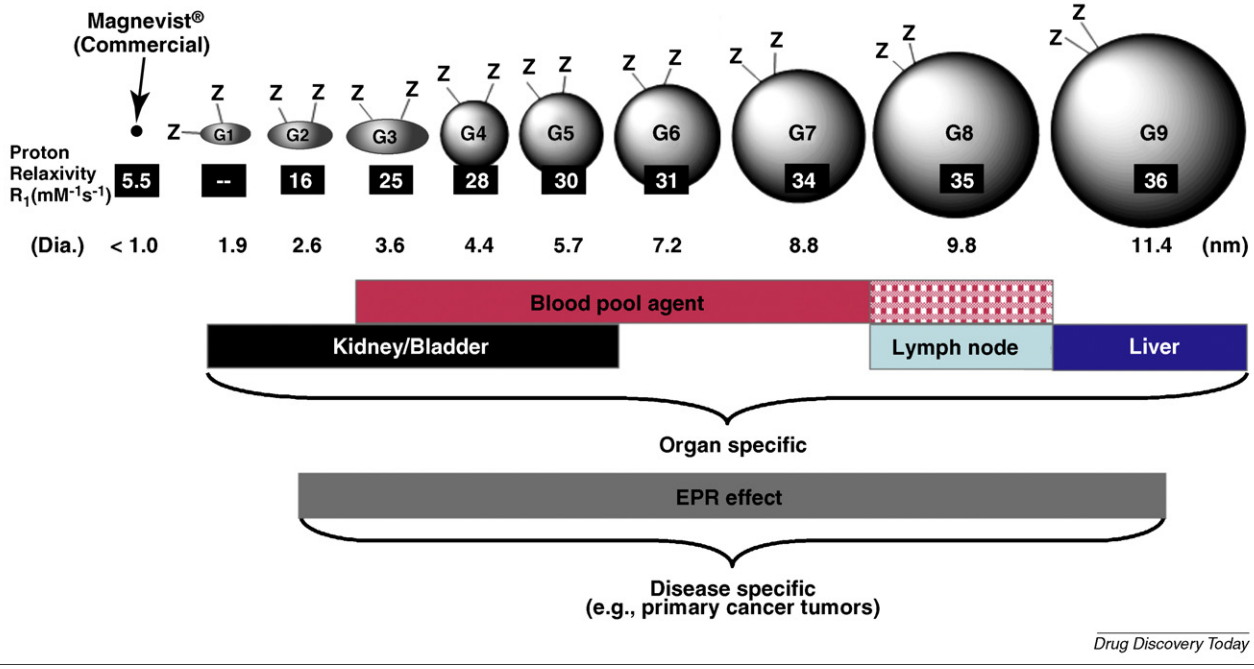

FIGURE 5

Overview of size-controlled PAMAM–DTPA (Gd) MRI contrast agents that demonstrate size-dependent blood agents, passive targeting of specific organs and the EPR effect.

drugs continues: Venuganti and Perumal [58] recently ranked permeability coefficient (Kp) of 5-fluorouracil assisted by dendrimers in the increasing order G = 3.5-COOH > G = 4-OH > G = 4-NH_2.

Dendrimers can be used to enhance the bioavailability of orally administered drugs that are hydrophobic, have low solubility and are P-gp substrates. Conjugation of propranolol to G = 3; PAMAM dendrimers increased its solubility and bypassed P-gp-mediated secretory efflux, thereby enhancing its absorptive transport [59]. Dendrimer surface modification with lauroyl chains substantially enhanced the oral absorption of conjugated drugs [60]. Clearly, dendrimers have exhibited great potential as solubility enhancers for insoluble drugs, and the use of PAMAM dendrimers for this application are described in Table 2. Complexation of furosemide (a nearly insoluble drug) with PAMAM dendrimers (<G = 4) improved drug solubility, as well as modulating release properties [61]. It is interesting to note that G = 0–3 PAMAM–NH_2 dendrimers offer better solubilization efficiency for insoluble Niclosamide than the β-cyclodextrins and hydroxypropyl β-cyclodextrins. Niclosamide release from G = 0–3; PAMAM–NH_2 was slower than the cyclodextrins because of the strong complex formation in the former, making them suitable candidates for controlled solid dosage forms [62]. In another instance, a 40% increase in aqueous solubility of sulfamethoxazole was achieved upon encapsulation of the drug in G = 3; PAMAM dendrimers [63].

The recent use of PAMAM dendrimers containing terminal amine groups (Starburst®) in antiperspirant deodorant compositions and for novel self-tanning cosmetic compositions has been reported to give improved efficacy and self-tanning activity when applied to skin [64].

Dendrimers – translation from research laboratory to market?

Translation from research and development to first human clinical trials has recently been realized for a series of anionic functionalized poly(L-lysine) dendrimers called Vivagel®. The gap between research and commercialization was bridged when Vivagel, developed by Starpharma (Melbourne, Australia) entered into phase II human clinical trials (see 'SPL7013 and HIV Infections' at http://www.clinicaltrials.gov). It is the first dendrimer-based product to have received Fast Track Status from the FDA under an investigational new drug application for the prevention of genital herpes (see 'SPL7013' at http://www.aidsinfo.nih.gov, October 6, 2008). A G = 4; poly(L-lysine)-based dendrimer with naphthalene disulfonic acid surface groups (i.e. SPL7013) is the active ingredient in Vivagel® [65,66]. Clinical efficacy of these products against HIV and genital herpes was demonstrated and reported recently [67]. Finally, its US-based wholly owned company, Dendritic Nanotechnologies Inc. (Mount Pleasant, MI), launched its first commercial Priostar® dendrimer-based product referred to as NanoJuice Transfection Kit [64].

The Stratus®CS AcuteCare™ NT-proBNP method, containing dendrimer-linked monoclonal antibody, was approved by the FDA to market as an *in vitro* diagnostic device for the quantitative determination of N-terminal pro-brain natriuretic peptide (NT-proBNP) in human plasma [65], whereas dendrimer-based MRI imaging agents (i.e. Gadomer series) are currently under investigation for clinical trials by Bayer Schering Pharma AG [68]. In closing, it should be mentioned that a popular *in vitro* transfection reagent – SuperFect®, based on modified Tomalia-type PAMAM dendrimers [69] – has been commercially available for nearly a decade from Qiagen, Germany.

These are just a few of the dendrimer-based nanomedicine products to reach commercial status. Many more are expected to follow.

Dendrimer properties of importance to nanomedicine

Nanomedicine focuses on the dynamic and structural roles of both biological and synthetic nanostructures or nanoassemblies that influence human disease and health. By definition, these constructs and assemblies include biological entities such as proteins, DNA and RNA, viruses, cellular lipid bilayers, cellular receptor sites and antibody variable regions crucial for immunology, to mention just a few. Critical Nanoscale Design Parameters (CNDPs) such as: (a) size, (b) shape, (c) surface chemistry, (d) flexibility and (e) architecture should be controlled to obtain a wide range of synthetic nanostructures. It is especially important when these CNDPs mimic and scale to the dimensions and features of biological structures or assemblies that influence human health and disease. Many of these CNDPs properties are manifested by dendrimers (Fig. 6) [35] and include (i) monodisperse nanosizes that scale with important bio-building block dimensions (i.e. protein, cellular lipid bilayer, virus and DNA and RNA) as a function of generation level; (ii) mathematically defined numbers of terminal surface groups (Z) suitable for bioconjugation of drugs, signaling groups, targeting moieties or biocompatibility groups that amplify as a function of generation level, according to the equation $Z = N_b N_c^G$; (iii) dendrimer surfaces that might be functionally designed to enhance or resist *trans*-cellular, epithelial or vascular biopermeability; (iv) well-defined interior void space within the dendrimer suitable for encapsulation (i.e. interior site isolation) of small-molecule drugs, metals or signaling groups (i.e. MRI or near-IR [NIR]). Incarceration of drugs within this void space reduces drug toxicity and allows controlled release. The interior is defined by the size and nature of the core (i.e. hydrophobic versus hydrophilic), as well as surface congestion, which increases with generation level; (v) positive biocompatibility patterns which are associated with lower generation (i.e. G = 1–5 for PAMAM dendrimers), anionic or neutral polar terminal surface groups compared with higher generation neutral apolar and cationic surface groups; (vi) non- or low-immunogenicity properties for most dendrimers functionalized with small-molecule or PEGylated/PEG-modified surface groups; (vii) the ability to statistically modify and optimize the number and/or ratio of dendrimer surface groups that influence biodistribution, receptor-mediated targeting, therapy dosage or controlled release of drugs from the dendrimer interior; and (viii) the ability to tune dendrimer mammalian excretion mode (i.e. urinary versus bile) as a function of nanoscale diameter (i.e. generation level) [35].

Concluding remarks

The high level of synthetic control over CNDPs (i.e. the size, shape, surface functionality and interior void space and so on) for dendrimers makes these nanostructures ideal vectors for both passive and active drug discovery and/or diagnostic imaging applications. Bioactive agents might be encapsulated into the interior, physically

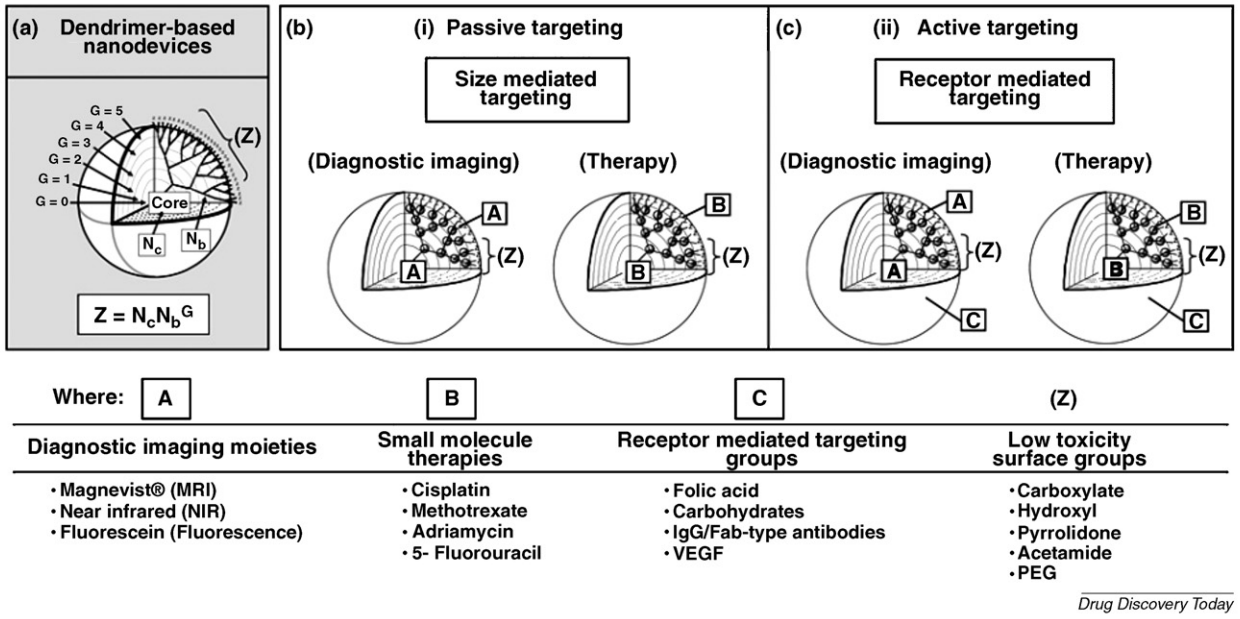

FIGURE 6

Dendrimer architecture and targeting modalities. **(a)** Illustration of general dendrimer architectural topology with the three architectural components: (i) core, (ii) interior and (iii) terminal surface groups (*Z*). **(b)** Passive size-mediated targeting: dendrimer-based diagnostic imaging and therapy delivery nanodevices involving (A) imaging moieties, (B) small-molecule therapy components and (*Z*) low-toxicity terminal surface groups. **(c)** Active receptor-mediated targeting: dendrimer-based diagnostic imaging and therapy delivery nanodevices involving (A) imaging moieties, (B) small-molecule therapy components, (C) receptor-mediated targeting groups and (*Z*) low-toxicity surface groups.

adsorbed or chemically attached to the dendrimer surface, with many options for tailoring vector properties to the specific needs of the active material and its therapeutic applications. Furthermore, the ability to select nanoscale-sized vectors with mathematically determined numbers of surface groups and well-defined interior void space allows systematic size adjustments to determine excretionary pathways while producing optimal ratios of targeting moieties, therapy and surface groups required in combination with desired solution behavior, excretionary pathway and acceptable toxicity margins. Finally, certain anionic surface-modified dendrimers are proving to function as safe and effective topical nanopharmaceuticals against HIV and genital herpes (http://www.starpharma.com/data/090803_VivaGel-Anti-HIV_%20and_Herpes_%20Activity_%20following_human_admin.pdf). These dendrimer-based nanopharmaceutics are in the final stages of human clinical testing in the FDA approval process. Hopefully, this brief review of dendrimer-based nanomedical applications clearly illustrates the potential of this new 'fourth architectural class of polymers' and reaffirms an even higher level of optimism for the future role of dendrimers in the emerging field of nanomedicine.

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