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Surface modified poly(amido) amine dendrimers as diverse nanomolecules for biomedical applications

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The unique properties of poly(amido)amine dendrimers such as nano size, multifunctional surface, ability to encapsulate and bind the guest molecules, efficient membrane transport and shelf-life stability have made them a promising carrier in drug delivery. The two key applications of dendrimers include increasing the solubility and sustained release of molecules, and drug targeting by grafting the cell-specific ligands on the surface. Despite their potential in drug delivery, inherent cytotoxicity, reticuloendothelial system uptake and hemolysis limit their use in clinical applications. Research groups have been working on surface modification methods to mitigate these problems. Herein we present a brief discussion on current surface modification approaches to: i) increase targeting efficiency; ii) increase the cellular permeability for enhanced absorption; iii) increase gene transfection efficiency; and iv) decrease the toxicity of the dendrimers, with a few classic examples. As the knowledge of relationship between the dendrimer surface chemistry and its mode of interactions with cell membrane is developed, so do the modifications of dendrimer structure to render them nontoxic, biocompatible, biodegradable and improve their pharmacodynamic and pharmacokinetic properties. Development of multifunctional dendrimers with each functional unit imparting a distinct characteristic feature such as target cell recognition, enhanced cellular transport, reduction in reticuloendothelial system uptake and stability in *in vivo* environment holds a great potential for future biomedical applications.

Keywords: cytotoxicity, drug delivery, drug targeting, gene transfection, PAMAM dendrimers, PD, PK, surface modification

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1. Introduction

Dendrimers are densely branched 'tree-like' macromolecules originating from a core molecule. Initially defined as "arborols" meaning monocascade spheres, dendrimers made their way into the rapidly growing field of polymer-based therapeutics [1,2]. Poly(amido)amine (PAMAM) dendrimers hold the distinction of being the first family of Starburst® dendrimers, which are completely synthesized by a sequence of two steps wherein concentric shells of dendritic units are produced around a central initiator core, the central initiator core being the ethylenediamine and concentric shells being *N*-(2-aminoethyl)acrylamide. This core-shell architecture grows linearly and the surface groups amplify exponentially at each generation. PAMAM dendrimers are biomimetic covalently fixed structures with interior void for filling and well-defined surface functionality. These outstanding

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features enable them to be utilized both as nanoscale containers as well as nano scaffolds [2,3]. Optical waveguide lightmode spectroscopy and atomic force microscopy techniques were used to investigate the kinetic and mechanistic aspects of interaction of the dendrimers with lipid bilayers. The mechanical model proposed to explain dendrimer generation and concentration effects on lipid bilayer showed adsorption to lipid bilayer and its removal as dominant mechanisms at nanomolar concentrations. The interaction kinetics of dendrimers with lipid bilayers was assumed to follow pseudo first-order kinetic model. These models have implications in biological applications of dendrimers such as cell transfection and cytotoxicity [4]. The interaction of PAMAM dendrimers with critical target sites such as vascular endothelium where most of the drugs or genes exert their effect has always been an alluring topic for biomedical researchers. PAMAM dendrimers were shown to extravasate through the microvasculature across endothelium into the surrounding interstitial tissue relatively quickly. The efficient extravasation of PAMAM dendrimers was attributed to its intrinsic charge bearing property as the glycocalyx layer lining the luminal endothelial surfaces, the walls of endothelial pores and the endothelial vesicles influence extravasation of charged molecules [5].

The biomimetic property and easily tailorable surface structure of the dendrimers together with identification of novel biomarkers for targeting the disease tissue have made PAMAM dendrimers interesting polymeric carriers to achieve higher binding affinity and selectivity. Nevertheless, the parent PAMAM dendrimers were also not devoid of any shortcomings, such as cytotoxicity, which have limited the commercial acceptance of PAMAM dendrimers. Thus, to efficiently transform the dendrimers into specific, selective, high affinity and biocompatible therapeutic nanoscaffolds, the need for surface modification has emerged. The main objective of this review is to comprehensively discuss the nuances of surface modification of dendrimers that has led to dendrimers of superior applicability in drug delivery research.

2. Surface modification for drug targeting

Targeted drug delivery to cancer cells or tumor vasculature is an alternative approach for effective cancer therapy. Like any other nanoparticulate system, targeting with dendrimers may be affected either by enhanced permeability and retention strategy or through receptor-mediated endocytosis using ligand-conjugated dendrimers. These effects are often desirable for cancer therapy as prolonged circulation and enhanced localization at the target would render drugs more effective at concentrations much below the threshold to cause side effects. Surface modification of dendrimers using different types of ligands including the small molecule ligands, for example, vitamins (folic acid, biotin etc.), cell penetrating peptides and antibodies against tumor associated antigens, have generated a variety of target-specific nanocarriers (**Table 1**).

2.1 Surface modification with small molecules

2.1.1 Folate-conjugated PAMAM dendrimers

Folate receptor (FR) expression is exponentially increased in many of human cancers and concordantly it is restricted in most normal tissues. Folic acid (molecular mass 441 Da) is usually transported into cells through FR-mediated endocytosis. It is evident from previous findings that when attached through its γ-carboxylic function, folic acid produces conjugates of almost any type, which can be endocytosed by FR expressing cells akin to what occurs with folic acid. Folic acid-conjugated PAMAMG5 amine-terminated dendrimers efficiently increased the toxicity of paclitaxel on human epidermoid carcinoma (KB) cells when compared with drug alone [6]. PAMAM dendrimers with folic acid as targeting moiety made contrast agents for MRI such as Gadolinium (^{153}Gd), more specifically localized to ovarian cancer. A fourth generation amine-terminated PAMAM dendrimer-folic acid conjugate when complexed with Gadolinium exhibited a 2700% increase *in vitro* binding to mouse erythroleukemia cells overexpressing FRs. This ^{153}Gd-folate-dendrimer complex exhibited 33% more accumulation in ovarian tumor xenografts as calculated in the form of percent contrast enhancement in ovarian cancer bearing nude mice [7]. Folate-conjugated PAMAM dendrimers were also investigated for applicability in boron neutron capture therapy, which requires selective delivery of ^{10}B to tumor cells. Boronated dendrimers conjugated with folic acid were shown to be specifically taken up by FRs overexpressed on KB cells. The high tumor uptake in mice bearing FR (+) 24JK-FBP (mouse sarcoma cell line transfected with human FR gene) was only observed with folate-conjugated- boronated PAMAMG3 dendrimers unlike their parent counterparts [8]. In addition to the specificity of FRs to tumors, it was demonstrated that folate conjugation can also be used to target inflammatory tissue. PAMAMG3.5 dendrimers when conjugated with folic acid using polyethyleneglycol (PEG 3350) as the spacer has shown to improve the targeting of indomethacin to inflammatory tissues and its pharmacokinetics *in vivo* [9]. Kukowska *et al.* (2005) have conjugated methotrexate (MTX) to PAMAMG5 dendrimers bearing folic acid as targeting moiety. Results revealed that MTX-conjugated dendrimer had significantly lower toxicity and 10-fold higher efficacy than free MTX [10].

2.1.2 Biotin-conjugated PAMAM dendrimers

Biotin is a growth promoter of the cells. Biotin levels were found significantly higher in some cancer cells as compared to normal tissue. The need for extra biotin by the rapidly proliferating cancer cells led several researchers to develop biotin-conjugated macromolecular carriers to enhance the uptake of drugs and macromolecules in tumor cells. Biotinylated PAMAMG5 dendrimers have shown increased uptake by HeLa (cervical cancer) cells compared with dendrimer alone. It was also shown that the enhanced uptake was specific to cancer cells expressing biotin receptors as biotinylated dendrimers

Table 1. Surface modified PAMAM dendrimers in targeting.

Dendrimer	Targeting ligand	Application	Ref.
PAMAMG5	Folic acid	Targeted therapy of paclitaxel to cancer	[6]
PAMAMG3.5	Folic acid	Targeting of indomethacin to inflammatory tissue	[9]
PAMAMG3 and G4	Mannose	Targeting of HIV-inactivating protein cyanovirin-N	[13,14]
PAMAMG2, G3 and G4	Arginine	Targeted non-viral gene delivery	[66,67]
PAMAMG5	Cholesterol	Targeted delivery of antisense oligos to HeLa cells	[100]
PAMAMG1 and G5	Biotin	Targeting drugs and contrast agents to cancer	[11,12]
PAMAMG4	Porphyrin	Phototriggered gene transfection	[20]
PAMAMG5	Herceptin	Targeted delivery of methotrexate	[25]
PAMAMG0 – G7	Anti-EGFR antibody hMAb425	Radioimmunotherapy	[28]
PAMAMG5	J591 antibody	Targeting prostate cancer	[26]
PAMAMG5	BH3 peptide	Induction of apoptosis in human carcinoma	[32]
PAMAMG6	Estrogen	Targeting estrogen receptors	[101]
PAMAMG5	LHRH peptide	Tumor targeting for early detection	[36]
PAMAMG5	RGD cyclopeptides	Targeting $\alpha_v\beta_3$ receptors on M21 melanoma	[29]
PAMAMG5	Tat peptide	Targeted delivery of antisense and siRNA oligonucleotides	[68]
PAMAMG3	CGS21680, an A_{2A} adenosine receptor agonist	Induction of signal transduction	[78]
PAMAMG4	Horseradish peroxidase	Biosensor for detection of glucose	[35]
PAMAMG5	EGF and cetuximab	Topical application for wound healing and targeting methotrexate to gliomas of brain	[33,34]

LHRH: Leutinizing hormone-releasing hormone; PAMAM: Poly(amido)amine; RGD: Arg-Lys-Asp.

failed to show increased uptake in normal cells such as HEK 293A (primary human embryonic kidney cells) and NIH/3T3 (mouse embryonic fibroblast cell line) [11]. In another study by Xu *et al.* (2007), biotinylated PAMAMG2 dendrimer with cystamine core along with lectin was successfully used as a targeting agent to deliver gadolinium-diethylenetriaminepentaacetate complexes (Gd(III)-1B4M-DTPA) for radiation therapy in a murine ovarian cancer model [12]. Our group prepared biotinylated PAMAM dendrimer-cisplatin conjugates and tested their cytotoxic activity in ovarian cancer cells (OVCAR-3, SKOV). Interestingly, amine terminated biotinylated PAMAM dendrimers loaded with cisplatin have shown highest activity against cisplatin-resistant ovarian cancer cells (A2780) when compared to cisplatin only (unpublished data).

2.1.3 *Mannosylated PAMAM dendrimers*

A wide variety of intracellular recognition events involve protein–carbohydrate interactions. The details of these interactions are needed to be explained clearly to promote efficient therapeutic outcomes. A variety of glycodendrimers with multivalent binding abilities have been developed to understand these interactions. Manα1-2Man functionalized G3 and G4 PAMAM dendrimers were investigated for their potential use to target HIV through cyanovirin-N. Cyanovirin-N is a HIV-inactivating protein, which utilizes HIV envelope proteins gp120 and gp41 as targets [13]. In an interesting study, mannose-conjugated PAMAM dendrimers were shown to bind with lectin two orders higher than simple methyl mannose indicating multivalent binding of the dendrimers [14].

2.1.4 PAMAM dendrimers modified by porphyrin for photodynamic applications

The principle of photodynamic therapy is to produce localized tissue necrosis by activating a photosensitizing drug in the target tissue with light of a specific wavelength matched to an absorption peak of the photosensitizer in the presence of molecular oxygen [15]. Photochemical internalization is a specific branch of photodynamic therapy used for the site-specific release of genes and drugs within cells. Photochemical internalization strategy has been used widely to release macromolecules such as dendrimer-doxorubicin conjugates, bleomycin, peptide nucleic acids and DNA [16-19]. Shieh *et al*. have developed a novel gene carrier by conjugating hydrophobic 5,10,15-tri(4-acetamidophenyl)-20-mono (4-carboxyl-phenyl)porphyrin (TAMCPP) to PAMAMG4 dendrimer. The cytotoxicity, phototoxicity and efficacy of the carrier were evaluated using HeLa cells as a model. Results indicated that G4-TAMCPP conjugate was efficiently taken up by HeLa cells and the conjugate accumulated in lysosomes/endosomes compartments through endocytic pathway. The most striking feature of this study was that the complex was able to achieve almost non-toxic transfection using 0.4 μM G4-TAMCPP plus irradiation, thus, proving the efficacy of surface modified PAMAM dendrimers as a photoinducible non-viral gene delivery system [20].

2.1.5 Metal complexes of PAMAM dendrimers

PAMAM dendrimers owing to their repeat monomeric characteristics have shown to be effective in increasing the circulation time of metals of biomedical interest compared to the monomeric chelates. To mention a few, Gd(III)-diethylenetriaminepentaacetic acid (used as contrast agent in magnetic resonance imaging), chromium (III) and Cobalt (III) [21,22]. Recently, PAMAM dendrimers were studied for local and controlled delivery of NO by functionalizing NO donor nitrosyl ruthenium complex. $[Ru^{II}(EDTA)NO]^-$ is a promising NO donor with low residence time in blood which is undesirable in some circumstances. $[Ru^{II}(EDTA)NO]^-$ pendant PAMAM dendrimers were successfully synthesized and were shown to be more effective (100%) than $[Ru^{II}(EDTA)NO]^-$ (89%) against *Trypanosoma cruzi* [23]. PAMAM dendrimers based on electrostatic interaction between charged surface groups and metal ion drugs such as cisplatin were developed to increase solubility of platinum-based drugs. The PAMAMG3.5 dendrimer with a COOH surface functionality was conjugated with cisplatin and it resulted in a complex with high drug solubility and slow release of platinum *in vitro*. PAMAM dendrimer-platinum conjugate was found highly effective in a murine melanoma model unlike simple cisplatin [24].

2.2 Surface modification with macromolecules

2.2.1 Antibody-conjugated dendrimers for targeting

PAMAM dendrimers had carved a niche for themselves as attractive carriers to make immunoconjugates as they were able to address problems associated with other immunoconjugates such as poor solubility and low binding affinity. Several groups have studied the conjugation of antibodies to PAMAM dendrimers for targeting applications. Shukla *et al*. (2008) have demonstrated that PAMAM dendrimer–herceptin conjugate selectively targets HER2 receptors expressed on MCA207 breast cancer cells without compromising the antifolate activity of MTX [25]. When PAMAMG5 dendrimers were conjugated with anti-PSMA (prostate-specific membrane antigen) antibody, J591, the resulting conjugate has shown specificity to PSMA positive prostate cancer cells (LNCaP) and was unable to bind to PSMA negative prostate cancer cell (PC3) confirming the targeting capability of J591-conjugated dendrimers. The conjugate also reduced non-specific interaction of the J591 antibody when compared with the antibody alone [26]. PAMAM dendrimers were also investigated for their advantages in radioimmunotherapy, as high specific activity is often necessary especially when using a radionuclide having a shorter half-life. PAMAMG4 dendrimers conjugated with OST7 (a murine monoclonal IgG_1) and 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethyl-ene triamine penta-acetic acid (1B4M) and labeled with In(III) and Gd(III) have shown very high specific activity and accumulation in the KT005 (human osteogenic sarcoma cell line) tumor bearing mice [27]. Moreover, PAMAM dendrimers provide a favorable derivatization pattern for antibodies without compromising the immunoreactivity of the antibody. Wängler *et al*. (2008) have investigated hMAb425 (anti-EGFR) antibody-conjugated PAMAM dendrimers with varying size and varying chelating agents per conjugation site. Results have shown that the size of dendrimers over a wide range did not affect the immunoreactivity but the number of chelating agents per conjugation site had influenced the immunoreactivity significantly [28]. Surprisingly, MTX-conjugated PAMAMG5 dendrimer previously grafted with herceptin has shown reduced *in vitro* toxicity. Co-localization experiments revealed that conjugate was localized in 1 h and had unusually long residence time (48 h) in lysosomal pocket explaining its reduced anti-folate activity *in vitro* [25].

2.2.2 Peptide/protein-conjugated dendrimers for targeting

Apart from myriad of applications of PAMAM dendrimers in targeting, dendrimers conjugated with specialized peptides and proteins have shown increased targeting efficiency both for drug and enzyme delivery. PAMAM dendrimers when chemoselectively ligated with Arg-Lys-Asp (RGD) cyclopeptide, the conjugate demonstrated the capability as a magnetic resonance contrast agent and as an optical agent [29]. Nanoscale dendritic RGD clusters synthesized with PAMAM dendrimers (3.5, 4 and 5 generations) have demonstrated potential as drug delivery devices to target tumor vasculature. Generation 5 PAMAM conjugate of RGD has shown greater uptake by cells expressing $\alpha_v\beta_3$ integrin receptors [30,31]. BH3 peptide has homology to the

anti-apoptotic Bcl-2 protein, which is known to be overexpressed in many cancers. BH3 peptide was shown to induce apoptosis nonspecifically in cancer as well as normal cells. To make this peptide target specific, folate-conjugated PAMAM dendrimers were attached to BH3 peptide and the apoptotic activity was investigated. The results demonstrated that BH3-conjugate of the dendrimer efficiently induced apoptosis specifically in KB cells overexpressing FRs [32].

EGF which is the ligand for EGF receptor (EGFR, ErbB) is chemically a 53 amino-acid peptide. It arbitrates signal events regulating cell proliferation, differentiation, angiogenesis and inhibition of apoptosis. EGFR is overexpressed in a variety of cancers such as ovary, lung and prostate. Thus, EGF-conjugated PAMAM dendrimers were considered for targeting cancer cells. PAMAMG5 dendrimers when conjugated with several EGF molecules served as EGFR superagonists. This superagonistic activity can be utilized in various conditions that require EGFR activation. For example, this conjugate can possibly be used to enhance wound healing by applying topically [33]. In another attempt to target EGFR, Wu *et al.* conjugated generation 5 PAMAM-MTX (G5-MTX) construct with Fc region of a monoclonal antibody cetuximab, which binds to EGFR and also EGFRvIII. This conjugate G5-MTX-cetuximab was localized sixfold higher in rats bearing EGFR expressed gliomas in comparison with rats bearing wild-type gliomas, thereby, showing specific molecular targeting to tumor. However, this conjugate did not have any significant effect on survival rates when compared with MTX alone [34].

PAMAM nanocomposites with enzymes were also investigated for the use in biosensing applications. Zeng *et al.* (2007) prepared enzymatic dendrimer hybrids by tethering looped horse radish peroxidase enzyme onto PAMAMG4. The hybrids have shown dense enzyme loading owing to higher availability of surface functional moieties (64 for PAMAMG4). The resultant sensors possessed a higher sensitivity for H_2O_2 detection and also have shown increased life time in comparison with conventional sensors [35]. Similarly, when hormones were conjugated to PAMAM dendrimers, they have shown a further targeting capability to the cancer cells. For example, leutinizing hormone-releasing hormone when functionalized on to PAMAMG5 dendrimer produced a stable conjugate that can be utilized for targeting, gene delivery and imaging [36].

2.2.3 *PAMAM dendrimers functionalized with other polymers*

Various polymeric nanodevices were investigated in biomedical research, but lack of increased surface functionality has created problems especially when high drug payload is required at single conjugation site. To overcome this drawback, PAMAM dendrimers were grafted with various polymers of biomedical interest. Liu *et al.* (2008) have fabricated a nanocomposite by grafting poly(methyl methacrylate) magnetic nanoparticles to the initial dendritic core, which resulted in PAMAM-grafted magnetic polymer microspheres. These conjugates on labeling with fluorescent probes were used as targetable nanodevices that can be easily traced *in vivo* [37].

In an approach to synthesize a novel nanoparticulate carrier to encompass a wide range of intracellular delivery applications, a combination of PAMAM dendrimer and natural polymer carboxymethyl chitosan was synthesized. This bipolymeric functional conjugate has proven to be biocompatible as assessed in L929 fibroblast and rat bone marrow stromal cells. Biochemical data proved that these hybrid nanoparticles have induced the osteogenic differentiation of rat bone marrow stem cells *in vitro*, thus, extrapolating the application of the conjugate for effective intracellular delivery of biomolecules to modulate the behavior of cells [38].

Zhang *et al.* (2007) have prepared poly (lactide-co-glycolide) (PLGA) microparticles with PAMAM dendrimers of generation 3, 4, 5 and 6 conjugated onto the surface of PLGA. The resulting PLGA dendrimer conjugates were then investigated for their gene transfection efficiency. PLGA-dendrimer microparticles significantly increased transfection efficiencies in comparison to unmodified PLGA. The increase in transfection efficiency of PLGA was reported to be owing to enhanced cellular uptake of plasmid DNA (pDNA) and increased endosomal buffering capacity of the PLGA-PAMAM complex. The former effect was owing to the higher ζ potential of PLGA-PAMAM microparticles in comparision with unmodified PLGA microparticles even after pDNA binding. For example, G3 PAMAM when conjugated to PLGA increased ζ potential of PLGA from -50 to +20 mV. Higher cationic ζ potential is known to enhance interaction of microparticles with negatively charged cellular membranes, which eventually lead to enhanced cellular uptake of pDNA. Moreover, conjugation of PAMAM dendrimers to PLGA reduced the generation dependent cytotoxicity of PAMAM dendrimers, thus, making these conjugates promising carriers for gene delivery [39]. A new approach in the field of anticancer drug delivery system known as modulatory liposomal controlled system (MLCRS) was recently reported. MLCRS consists of a liposomal formulation composed of lipids and of drug-dendrimer complex. Liposomal formulation of doxorubicin, which is present in market, involved use of pH gradient method for encapsulation of doxorubicin. Using MLCRS approach, liposomal formulations were developed by incorporating doxorubicin-PAMAM complex. The liposomes with PAMAM-doxorubicin conjugates exhibited enhanced release rate and better cytotoxicity when compared with conventional liposomes [40].

Doxorubicin covalently conjugated to PAMAM dendrimers through pH sensitive and insensitive linkers was also investigated for cancer therapy. The PAMAM-hydrazone-doxorubicin conjugate resulted in more nuclear accumulation of drug eventually leading to more cytotoxicity to the cancer cells [19]. In one of the most recent reports, Sarkar and Yang (2008) have demonstrated that encapsulation of anastrazole (poorly soluble anticancer agent used to treat breast cancer in post

menopausal women) into stealth PEG-PAMAM dendrimers has increased aqueous solubility of the drug with extended release property [41,42]. Similarly, when MTX was encapsulated into PEGylated PAMAMG3 dendrimer, extended release of MTX from dendrimer was observed as compared to unencapsulated drug [43]. Apart from enhanced solubility and extended release, PEG-PAMAM dendrimers also confer enhanced stability to peptides and small molecule drugs. PAMAM-PEG has shown improved stability of a model peptide *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide when subjected to catalyze hydrolysis by α-chymotrypsin [44]. Doxorubicin when loaded into PEGylated polyester dendrimers exhibited enhanced *in vitro* stability and was found to deliver as much doxorubicin as clinically used PEGylated liposomal formulation DOXIL [45].

3. Enhancement of oral delivery by surface modification of PAMAM dendrimers

The route of drug administration is of prime importance when it comes to patient compliance. Many polymeric drug carriers were investigated for enhancing oral bioavailability of poorly absorbed drugs. Unlike other polymers, PAMAM dendrimers were shown to permeate across epithelial barriers owing to their nano size and charge-mediated interaction with the membrane [46], making them suitable carriers for oral drug permeability. The mechanism through which PAMAM dendrimers enhance oral permeability is a combination of paracellular transport and an energy-dependent process such as adsorptive endocytosis [47]. It was demonstrated that PAMAM dendrimers exert their effect by modulating the tight junctional proteins occludin and actin [48]. Furthermore, PAMAM dendrimers are not substrates of p-glycoprotein (P-gp) efflux system; thus, they have an extra advantage over conventional polymers [49].

Our research group has investigated PAMAM dendrimers tethered with polyamines arginine and ornithine. It has been documented that polyamines are involved in growth and differentiation of gastrointestinal cells [50]. These polyamines were assumed to be transported into the cell by polyamine transporter protein system. Because of their intestinal permeation enhancement effect, and their absorption by carrier-mediated transport, we hypothesized that conjugation of these polyamines to PAMAM dendrimers increase their permeability across epithelial cell monolayers. Arginine and ornithine conjugated PAMAM dendrimers have shown increase in permeability coefficient (Papp) $(0.46 \pm 0.07) \times 10^{-6}$ and $(0.63 \pm 0.13) \times 10^{-6}$ cm/s, respectively, with concomitant reduction in transepithelial electric resistance when compared with parent PAMAM $(0.34 \pm 0.01) \times 10^{-6}$ cm/s. Results indicate that the increase in permeability with the surface-modified dendrimers may be due to disruption of intercellular tight junctions as one of the mechanisms [51]. Similar results were obtained with IPEC-J2, a primary cell line derived from jejunal epithelia isolated from neonatal, unsuckled piglet [52], suggesting that the polyamine-conjugated dendrimers have greater potential for mucosal targeting of vaccines and enhancing oral delivery of drug and macromolecules. We and several others have demonstrated increase in cytotoxicity of PAMAM dendrimer with increase in generation number [53,54]. To mitigate the problem, Jevprasesphant *et al.* (2003) have prepared PAMAM lauroyl chloride (C_{12}, a medium-chain fatty acid)-conjugated PAMAM dendrimers and loaded naproxen, which has low aqueous solubility. The resulting dendrimer-drug complex has shown enhanced solubility, stability and reduced toxicity [55]. The complex exhibited a significant increase in permeability of naproxen when compared with naproxen itself and stability of naproxen in plasma and liver homogenate was also found to be improved [56].

Of late, several studies have documented enhanced oral permeability of drugs either covalently conjugated or encapsulated in PAMAM dendrimers (**Table 2**). Doxorubicin, an anticancer drug when encapsulated in PAMAM dendrimer has led to a staggering ~ 700-fold increase in bioavailability as compared to free doxorubicin [57]. 7-Ethyl-10-hydroxy-camptothecin (SN-38), a potent topoisomerase I poison, has antitumor activity but suffers from poor oral bioavailability. SN-38, when complexed with G4 PAMAM dendrimers, showed a 10-fold increase in permeability and > 100-fold increase in cellular uptake was observed as compared to free SN-38 [58]. PAMAMG1 dendrimer-based prodrugs for water insoluble P-gp substrate, terfenadine, were synthesized using succininc acid or succinyl diethylene glycol as a linker/spacer. Results indicated that terfenadine when covalently bonded to PAMAMG1 increased permeability across caco-2 monolayers. The mere presence of PAMAMG1 in transport medium had no effect on permeability of terfenadine [59].

4. Surface engineered PAMAM dendrimers as gene delivery vectors

Dendrimers have largely proved themselves as efficient carriers for non-viral gene delivery owing to their small size and ability to condense DNA thereby protecting DNA from *in vivo* environment. This is substantiated by the fact that although the utilization of dendrimers as gene delivery carriers is just a few decades old, there are already commercial products available as gene transfecting agents. Polyfect® and Superfect® are the two commercially available activated dendrimers as gene transfecting agents wherein Polyfect® has intact dendrimer and Superfect® has fractured dendrimer as their component [60]. These dendrimers have primary amine groups on their surface which bind to the DNA and aid in condensing DNA into nanoscale complexes (called as dendriplexes) and eventually enhance the cellular uptake of DNA, while the tertiary amine groups, which are buried in the deep core of dendrimers acts as proton sponge in endosomes and enhance the release of DNA into the cytoplasm.

Table 2. Examples of surface modified dendrimers in enhancement of oral permeability.

Dendrimer	Drug/ligand	Mode of attachment	Result	Ref.
PAMAMG4	7-Ethyl-10-hydroxy-camptothecin (SN-38)	Covalent conjugation	Enhanced oral bioavailability	[58]
PAMAMG3	Doxorubicin	Encapsulation	Enhanced oral bioavailability	[57]
PAMAMG2 – G4 and G2.5 – G3.5	Lauroyl chloride	Covalent conjugation	Reduced toxicity and enhanced oral permeability of dendrimers	[55]
PAMAMG4	Arginine and ornithine	Covalent conjugation	Reduced toxicity and enhanced oral permeability	[51,52]
PAMAMG0	Naproxen	Covalent conjugation	Enhanced oral permeability	[56]
PAMAMG1	Terfenadine	Covalent conjugation	Enhanced oral permeability	[59]
PAMAMG2 and G4	Acetyl groups	Covalent conjugation	Reduced toxicity and enhanced oral permeability of dendrimers	[91]
PAMAMG3, G4 and PAMAM2.5, 3.5 and 5.5	^{125} Iodine	Encapsulation	Enhanced oral bioavailability	[102]

PAMAM: Poly(amido)amine.

Although dendrimers can carry high gene load, their major limitations for *in vivo* application are low transfection efficiency, lack of target specificity and limited transport into the nucleus of the target cells. Several surface engineered PAMAM dendrimers were designed to overcome these problems (**Table 3**). Amino-acid conjugates of dendrimers were proven to be useful in enhancing the transfection efficiency of dendrimers. Kono *et al.* (2005) have demonstrated that PAMAMG4 dendrimers having phenylalanine and leucine residues at surface increase the transfection ability of dendrimers. These hydrophobic amino acids were expected to increase the transfection activity by virtue of synergistic activity of proton sponge effect and hydrophobic interactions with cells. Interestingly, the phenylalanine conjugated-dendrimers have shown a twofold increase in transfection when compared to unmodified dendrimers, whereas leucine conjugated dendrimers did not show any significant increase. The reason for this difference in transfection activity between the two amino acids was attributed to their relative hydrophobicities as phenylalanine was found to be more hydrophobic than leucine [61]. Protein transduction domains or membrane translocalization signals contain positively charged aminoacids, arginine and lysine. Surface modification of PAMAM dendrimers by conjugating with L-arginine has shown higher transfection efficiency when compared to PAMAM dendrimers and PAMAM-lysine conjugate. Although it is not clear if the conjugates enter into cell membranes by an endocytic or nonendocytic pathway or direct penetration into membranes, enhanced cellular internalization has been reported by many research groups [62-66]. It should be noted that attachment of arginine to PAMAM dendrimer through a biodegradable ester bond has better biodegradability when compared with arginine bound with an amide bond [67]. PAMAMG5 dendrimer when conjugated with Tat peptide, a cell penetrating peptide, exhibited an effective delivery of antisense oligonucleotides; however, the conjugates were ineffective in delivering siRNA [68].

Because of multi surface functionality, it is relatively easy to conjugate PAMAM dendrimers with ligands, which would enhance gene delivery potency and also reduce the cytotoxicity of dendrimers. PAMAMG5 dendrimers when conjugated with poly (ethylene glycol) (PEG-3400), resulted in 20-fold increase in transfection efficiency compared with PAMAM alone. Cytotoxicity of these new conjugates was also very low thus making it a promising transfecting agent [69]. In another approach, quaternary ammonium groups were introduced to PAMAM dendrimers, a process called "quaternization", mainly to reduce cytotoxicity by imparting hydrophilicity to the PAMAM dendrimers. When PAMAMG4 was quaternized and subjected to transfection in human embryonic kidney cells (HEK 293T), results have

Table 3. Applications of surface modified PAMAM dendrimers as gene delivery systems.

Dendrimer	Ligand	Gene	Result	Ref.
PAMAMG5, G6 and G7	None	Human bone morphogentic protein-2	*In vitro* differentiation of mesenchymal stem cells	[103]
PAMAMG4	Quaternary amine groups	Luciferase	Low cytotoxicity and comparable transfection ability with unmodified PAMAM	[70]
PAMAMG6	None	Calf thymus DNA	Transfecting agent	[104]
Superfect®	Not reported	IL-2 gene	Immunotherapy of Ewing tumors	[105]
Polyfect®	Not reported	Primary cultures of astrocytes from cerebral hemispheres	Role of JNK1 in glia cell inflammation	[106]
PAMAMG4 to G8 with a Trimesyl core	None	Luciferase	Increased transfection efficiency	[107]
PAMAMG4	Porphyrin	Enhanced green fluorescent protein plasmid DNA	Phototriggered gene transfection and reduced toxicity of PAMAM	[20]
PAMAMG4	Internally quaternized and surface acetylated	Fluorescent RNA duplex-siRNA labeled with NuLight DY-547	Enhanced delivery of siRNA to A2780 ovarian cancer cells	[108]
PAMAMG2, G3 and G4	Arginine	Luciferase	Enhanced transfection in HUVEC cells	[66]
PAMAMG3, G4 and G5	Phosphorus	β-gal	Enhanced transfection	[109]
PAMAMG3, G4, G5 and G6	Poly (D,L-lactide-co-glycolide)	Luciferase	Increased transfection, reduced generation dependent toxicity	[39]

PAMAM: Poly(amido)amine.

shown that quaternized PAMAM dendrimers increased cell viability by ~ 15 – 20% when compared with parent PAMAM dendrimers [70].

Polyamines (ornithine, putrescine, spermidine and spermine) rapidly take up actively dividing cells such as cancer cells and are also well known for condensing and packaging DNA into compact forms [71-74]. It was assumed that synthesis of the dendrimer that has surplus of spatially oriented polyamine molecules might contribute to enhanced uptake by cells, resulting in high transfection efficiency. Accordingly, ornithine-conjugated with PAMAMG4 dendrimers have shown high transfection efficiency (59.83 ± 3.78%) as compared to parent PAMAMG4 dendrimers (25.78 ± 5.28%) in HEK 293T cells (**Figure 1**). Cytotoxicity of ornithine-conjugated dendrimers was comparable to that of PAMAM dendrimers and may be considered safe ≤ 50 µg/ml (**Figure 2**).

5. PAMAM dendrimers modified with ligands for molecular sensing and signal transduction

Contrasting agents are widely used for the early detection of life threatening diseases, such as cancer, congenital heart disease and a battery of other vascular diseases. However, currently approved low molecular mass contrasting agents have rapid body clearance [75]. To overcome this problem, protein-based macromolecular contrasting agents are developed but immunogenicity is their limitation. PAMAM dendrimers are non-immunogenic, can be easily tailored with targeting ligands and possess the ability to harbor large number of metal ions, which together will result in improvement of image contrast resolution from centimeter to as low as ~ 100 µm [76]. This would enhance the detection of various anatomical structures previously undetected. Myc *et al.*

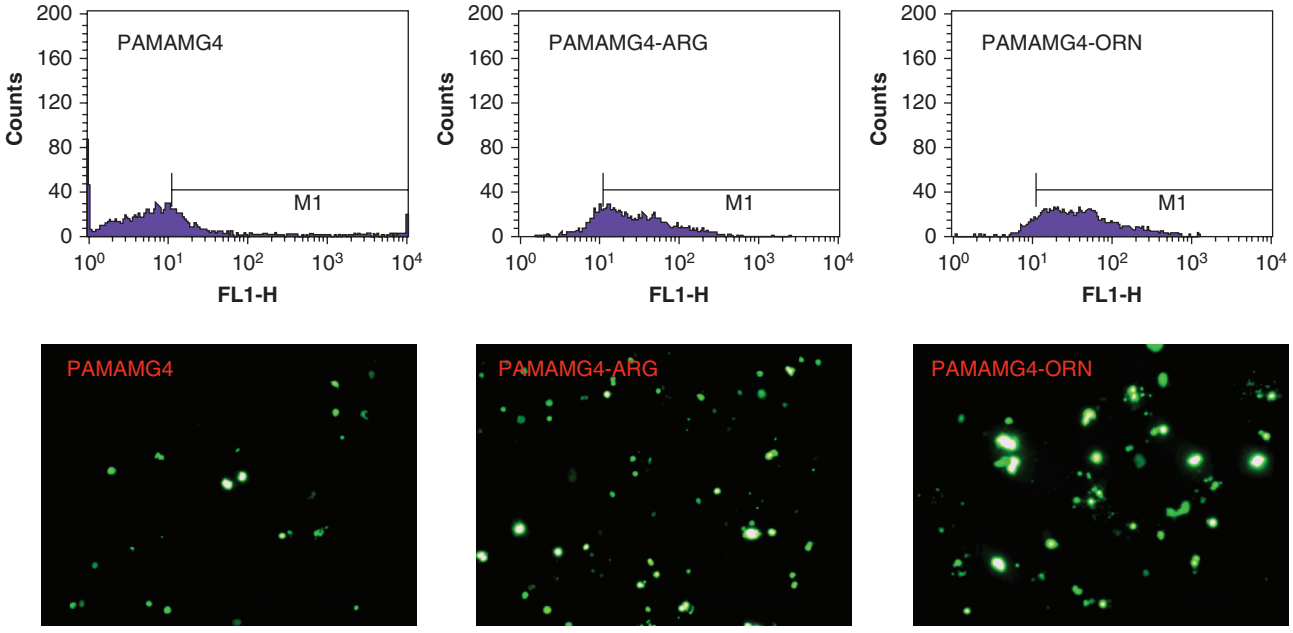

Figure 1. Transfection efficiency of arginine- and ornithine-conjugated PAMAMG4 dendrimers. Transfection efficiency of arginine-conjugated PAMAMG4, ornithine-conjugated PAMAMG4 and parent PAMAMG4 in HEK 293T cells determined by flow cytometry (upper panel) and their corresponding fluorescent micrographs (lower panel).
HEK: Human embryonic kidney cell; PAMAMG4: Poly(amido)amine generation 4.

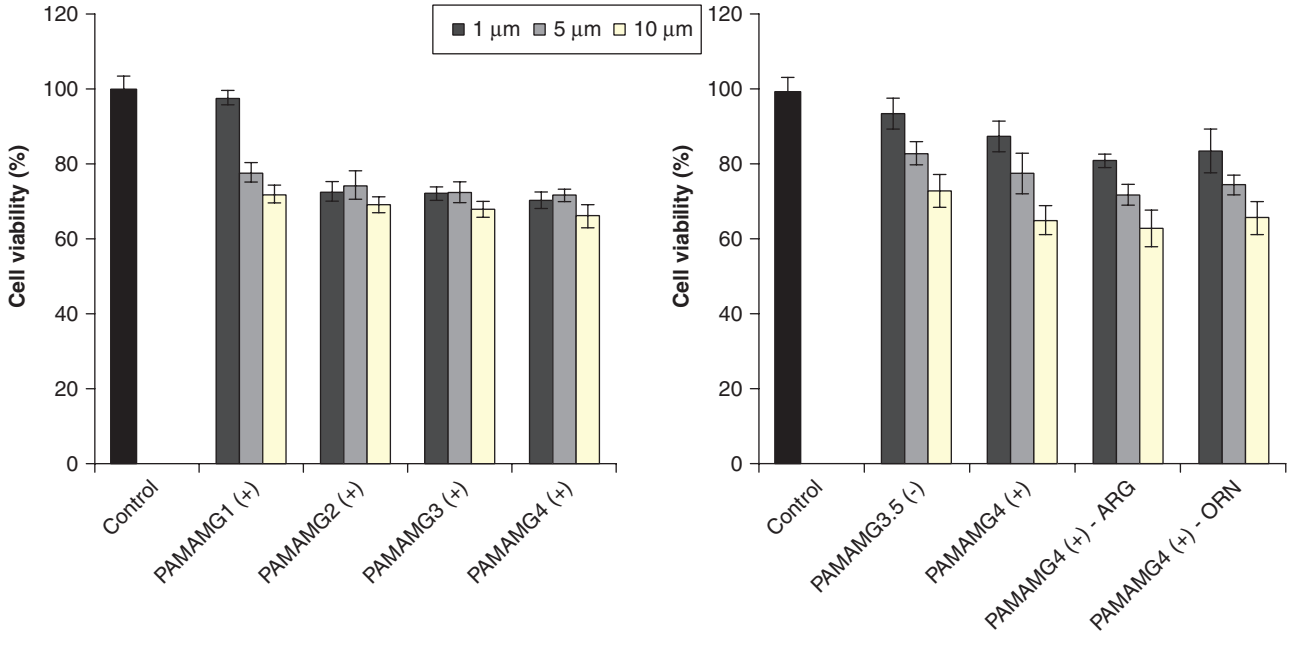

Figure 2. Cytotoxicity of dendrimers in HEK 293T cells. HEK 293T cells were co-incubated with dendrimers for 24 h at concentrations of 1, 5 and 10 µM of different dendrimers separately in quadruplicate. Cytotoxicity of dendrimers was assessed by a methylthiazoletetrazolium assay. Left panel: Percent cell viability after incubation with different generation of PAMAM dendrimer. Right panel: Percent cell viability comparison of PAMAMG3.5, PAMAMG4, arginine-conjugated PAMAMG4 and ornithine-conjugated PAMAMG4 dendrimers.
HEK: Human embryonic kidney cell; PAMAM: Poly(amido)amine.

(2007) have reported a bifunctional PAMAMG5-based nanodevice that comprised of folic acid as targeting moiety and fluorescence resonance energy transfer reagent "PhiPhiLux G_1D_2" as an apoptosis-detecting agent. The results have shown that the nanodevice specifically detects apoptosis in targeted cells unlike un-conjugated apoptotic sensor [77].

In another study, DITC (4-isothiocyanatophenylaminothi ocarbonyl)–APEC (-2-[4-(2-aminoethylaminocarbonylethyl) phenylethylamino]-5′-N-ethylcarboxamidoadenosine, an A_{2A} adenosine receptor specific agonist, was conjugated to PAMAMG3 dendrimer by forming a thiourea linkage [78]. The conjugate was then loaded with fluorescein and *in vitro* binding to human adenosine receptors was performed in Chinese hamster ovary and HEK-293 cells. The functionalized PAMAM dendrimers have shown increased affinity at A_3 subtype of adenosine receptor. Furthermore, these conjugates have shown an efficient inhibitory effect on platelet aggregation stimulated by ADP [78]. Alexander *et al*. (2003) have developed PAMAM dendrimer-based biometric nanoprobes by incorporating Gd^{III} for detection of the tumor vascular permeability. PAMAM-Gd^{III} complexes using generations 4, 6, 8 PAMAM dendrimers were synthesized and tested for their activity in mice bearing squamous cell carcinoma VII tumors. G8-Gd complex has shown more stable enhancement of signal over time when compared with G6-Gd, while G4-Gd nanoprobe exhibited a rapid clearance from body with rapid decreasing signal [79].

6. Enhancement of biopharmaceutical properties of drugs by PAMAM dendrimers

A majority of new drug candidates fail during the development process because of unfavorable biopharmaceutical properties such as high molecular mass, poor water solubility, toxicity, low permeability and poor stability. These properties, especially solubility and release profile of a drug, may be improved by incorporating the drug into a dendrimer in different ways: i) complex formation by electrostatic interactions; ii) covalent attachment; iii) conjugation through linker; and iv) interaction through van der Waals forces (**Table 4**). It has been demonstrated that drugs conjugated through a linker provide a slow and sustained release as compared to electrostatic or covalent complexation.

Apart from the anticancer drugs discussed earlier, NSAIDs are the most widely investigated drugs using dendrimers as drug delivery vehicles. Many of the effective NSAIDs with well-known anti-inflammatory, antipyretic and analgesic properties have low solubility in water and cause local or systemic disturbance in the gastrointestinal tract. Ketoprofen, a propionic acid derivative, when complexed with PAMAMG5 dendrimer showed slower release rate of drug when compared to drug alone. This complex was also investigated for *in vivo* pharmacodynamic (PD) and pharmacokinetic (PK) activity in mice. In this study, ketoprofen and its complex with PAMAM dendrimers were administered orally in form of solution in edible oil to Kunming mice. Prolonged PD and PK activity was observed with dendrimer-ketoprofen complex [80]. Similar results were observed when ibuprofen was complexed with PAMAMG4 dendrimer. This paper reported *in vitro* release and cellular uptake studies of dendrimers-ketoprofen complex. Interestingly, the complex apart from showing extended release also showed increased cellular internalization in A549 (carcinomic human alveolar basal epithelial) cells demonstrating the targeting capability of PAMAM dendrimers [81].

In another study involving *in vitro* cellular uptake studies, methylprednisolone, a corticosteroid, was covalently conjugated with PAMAMG4 dendrimer using glutaric acid as spacer. The conjugates exhibited increased cellular internalization with intact therapeutic activity when compared with free methylprednisolone [82]. Other NSAIDs such as 5-aminosalicylic acid, naproxen and indomethacin were also complexed with PAMAM dendrimers and showed improved oral bioavailability and also enhanced pharmacological activity [83-85].

PAMAM dendrimers because of their intrinsic mucoadhesive properties were investigated for their potential as drug carriers for antibacterial agents such as triclosan and quinolones (e.g., nadifloxacin and prulifloxacin). Complexation of these drugs with PAMAM dendrimers has shown significant improvement in their solubility characteristics and thereby antibacterial activity [86,87]. Those mentioned above are examples of some of the PAMAM dendrimer drug complexes of major therapeutic classes. The discussion of entire classes of drugs complexed with dendrimers is out of scope of this review. However, **Table 4** briefly explains all types of PAMAM dendrimer drug complexes reported so far.

7. Effect of surface modification on PK properties and toxicity of dendrimers

The loading ability of drugs/biologically active molecules in the dendrimers depends on dendrimer generation number. While the solubility, PD and PK behaviors of drugs in dendrimer-based formulations depend on dendrimer surface components, the release rate of the drug is controlled by the type of the linker between the dendrimer and the drug.

The encapsulation, conjugation or complexation of drug to the dendrimer affects its solubility and release rate of the drug. The release rate of a drug is a crucial factor controlling the PK parameters (V_d, AUC, $t_{1/2}$, C_{max}, t_{max}) and thereby therapeutic outcomes. Several drugs have shown improved pharmacological activities when used as dendrimer-drug complexes as compared to free drugs. However, for those drugs that have conformational changes with varying pH conditions, simple amine-terminated PAMAM dendrimers may not be the right choice (for example camptothecin and pilocorpine). As with any other nanoparticulate system, PAMAM-NH2 dendrimers accumulate in liver with only ~ 1% of injected drug in blood after intravenous administration, while

Table 4. Surface modified PAMAM dendrimers in drug delivery.

Dendrimer	Drug	Mode of conjugation	Results/indications	Ref.
PAMAMG2, G3 and G2.5	Enoxaparin	Complexation	Enhancement of pulmonary absorption	[110]
PAMAMG0 – G3	Triclosan	Covalent conjugation	Enhanced solubility and stability	[86]
PAMAMG3 and G4	Anastrozole	Complexation	Improved solubility and extended release	[42]
PAMAMG5	Methotrexate	Covalent conjugation	Targeting to cancer cells	[25]
PAMAMG2.5 and PAMAMG4-OH	Methylprednisolone	Covalent conjugation	Improved stability and targeting to cells	[82]
PAMAMG3.5	Cisplatin	Electrostatic conjugation	Improved targeting to cancer	[24]
PAMAMG3	Niclosamide	Complexation	Enhanced solubilization and prolonged release	[111]
PAMAMG3	Sulfamethoxazole	Complexation	Sustain release of sulfamethoxazole	[112]

PAMAM: Poly(amido)amine.

carboxy-terminated PEGylated dendrimers exhibit longer circulation time. As most of the NSAIDs contain carboxylic groups, PAMAM-NH2 dendrimers may be considered as the suitable vehicles as they improved pharmacokinetics (AUC, bioavailability and targeting efficiency in the inflamed regions) of these drugs (80.83 – 85). Moreover, PAMAM dendrimers prolonged the retention of the drugs (NSAIDs) at inflamed site in spite of presence of lymphatic drainage. Similarly, improved pharmacokinetics was also observed with other drugs, such as primaquine phosphate and 5-fluorouracil. If the dendrimer-drug complexes exhibit superior pharmacological activity *in vitro*, but with poor PD and PK performance, the complexes may be either administered as local delivery systems or they may be conjugated with targeting ligands (folic acid, galactose) or PEGylated to alter the PD/PK profile of these complexes.

Surface modification of PAMAM dendrimers greatly influences PK properties of dendrimers and this in turn would result in modification of PK profile of encapsulated drugs. According to Kaminskas *et al.*, the initial half-lifes of poly-L-Lysine dendrimers were greatly increased (from 3 – 4 to 30 – 40 min) even with modification of dendrimers with short chain length PEG (200 Da). This increase in circulation time of PEGylated dendrimers was ascribed to ability of PEG to cap cationic charge of dendrimers, reduce opsonization and phagocytosis by reticuloendothelial system and reduce proteolysis [88]. Folate-PAMAM dendrimer conjugates resulted in significant increase in half-life (> 152%) along with increased AUC ($AUC_{0-\infty}$ ((μg h)/ml) PAMAM-indomethacin: 91.93 ± 10.35, folate-PAMAM-indomethacin: 166.58 ± 0.20) and reduced clearance (Cl (ml/(min kg)) PAMAM-indomethacin: 0.61 ± 0.07, folate-PAMAM-indomethacin: 0.33 ± 0.00). Tissue distribution studies revealed that when compared with parent PAMAM, folate-conjugated PAMAM dendrimers exhibited reduced uptake by the reticuloendothelial system organs (spleen, liver etc.). Folate-PAMAM conjugate in this context was also shown to get accumulated in inflamed paw demonstrating the ability of surface modified dendrimers to target arthritic tissues [89]. *In vivo* studies with doxorubicin-loaded PAMAM dendrimers after oral administration to rats revealed that AUC values of PAMAM-doxorubicin complex were 200-fold higher than those obtained with the doxorubicin solution. This increase in AUC is attributed to the capability of PAMAM-doxorubicin complex to bypass drug efflux by P-gp and cytochrome $_P$450 (CYP) metabolism [57]. Dendrimer-based drug delivery systems are in their infancy and currently limited PK and PD data are available. Although several cell culture and animal experiments have demonstrated the efficacy of drug-loaded dendrimers, two main obstacles that prevent these formulations from preclinical success are rapid clearance and toxicity. Future efforts in this field should be directed to development of dendritic scaffolds with long circulation time and biodegradation properties.

PAMAM dendrimers are useful in improving the poor pharmaceutical properties, but their generation dependent toxicity is undesirable. To overcome this drawback, dendrimers were surface modified with various moieties. Initially, attempts to reduce toxicity by D'Emanuele *et al.* involved attachment of lauroyl side chains and PEG molecules to PAMAM dendrimers. For instance, attachment of six lauroyl side chains to cationic dendrimers resulted in sevenfold increase of IC_{50} values of these cationic dendrimers [55]. Similarly, attachment of four PEG chains increased the IC_{50} of cationic dendrimers by six times [90]. Esterification of

PAMAM dendrimers resulted in lower toxicity to primary cells such as HUVEC and primary rat aorta vascular smooth muscle cells. This reduced toxicity was owing to biodegradable nature of ester moieties attached to PAMAM dendrimers [67]. Acetylation of PAMAM dendrimers is another valuable approach to reduce the cytotoxicity of PAMAM dendrimers. Acetylation resulted in > 10-fold reduction in cytotoxcity towards Caco-2 cells while maintaining permeability across cell monolayers [91]. PEGylation of PAMAM dendrimers resulted in stealth dendrimers capable of very high biocompatibility. After 24 h incubation with endothelial cells, the PEG-PAMAM dendrimers exhibited a 40% increase in cell viability as compared to native PAMAM dendrimers [92].

PEG is a water soluble, nontoxic and nonimmunogenic polymer widely used in drug delivery as a structural modifier [93-95]. As discussed earlier, the surface chemistry of dendrimers is very much responsible for its inherent properties such as toxicity, interactions with foreign objects, routes of cellular uptake and intracellular fate. Thus, to optimize the pharmacological effects of dendrimers and to reduce their toxicity, PEGylation approach is considered. PEG may be attached to dendrimers either by forming covalent or electrostatic bonds [69,96,97]. In a recent report, amine-terminated PAMAMG3 dendrimer was attached with PEG groups of various sizes (i.e., M_n = 550, 750 and 2000) and compared with acetylated conjugate. The toxicity of PEG-dendrimer conjugates (PEG M_n = 550, 750) was two to ninefold lower than acetylated dendrimers (14 acetyl groups) [98]. It was also demonstrated that the degree of PEGylation of the dendrimers (3PEG-PAMAMG3, 8PEG-PAMAMG3, 10PEG-PAMAMG4 and 21PEG-PAMAMG4) affects the cytocompatibility of the dendrimers. Furthermore, the degree of PEGylation can be kept low (3PEG-PAMAMG3 and 10PEG-PAMAMG4) without compromising on biocompatibility [92]. Wang *et al*., in a recent paper reported that mechanism of reduced cytotoxicity by attachment of PEG was due to the reduction of PAMAM induced apoptosis, which in turn was the result of two synergistic actions namely attenuation of reactive oxygen species and inhibition of MMP induced by PAMAM [99]. In summary, PEG-PAMAM dendrimer conjugates can emerge as versatile drug delivery nanoscaffolds with desired pharmacological properties.

8. Expert opinion

The two major applications of dendrimers in pharmaceutical research are: i) drugs may be either incorporated into the dendrimers to form inclusion complexes or drugs may be covalently linked to the terminal groups of the dendrimers; and ii) targeted drug/radionuclide/gene therapy may be achieved by conjugating target-specific ligands to the dendrimer. The most widely sought objective of modern drug delivery research is to apply molecular and bioanalytical techniques to identify novel biomarkers for a variety of diseases and use those markers as ligands for targeted delivery of drug-loaded nanoparticles. The advantage of using the dendrimers in realizing this objective is that several copies of the ligand molecule may be easily attached to the dendrimers surface thereby increasing the avidity and affinity of the dendrimer to the target molecules.

The important parameters used in the assessment of nanoparticles for biomedical applications are polydispersity, multifunctional surface for enhanced signal generation, stability and their biocompatibility. Although PAMAM dendrimers have met the first three standards above, toxicity has been the main concern for *in vivo* applications. It is well documented that cationic dendrimers are more toxic than dendrimers with neutral or negative surface charge (OH or COOH) and their toxicity increases with generation number. The main objective of the present dendrimer research is to either modify the surface properties of the dendrimers or synthesize altogether new dendrimers with biocompatible molecules. Several techniques were reported to reduce the toxicity of the dendrimers, for example, acylation of the terminal cationic groups has been shown to reduce the toxicity. For many years, glyco-dendrimers containing various surface moieties such as glucose, mannose, lactose, sialic acid and sulfated sugars have been synthesized for potential antiviral and antimicrobial properties, which are the basis for the clinical testing of VivaGel™ StarPharma, an antiviral formulation containing generation 3 polylysine dendrimer with napthyl disodium sulfonate on the surface.

Although safety concerns of dendrimers are very well justified, it is reasonable to expect that previous preclinical and clinical experience gained during the development of polymer therapeutics combined with growing knowledge of novel natural biocompatible polymers can be used in the selection of polymers with proven safety profile to ensure development of safe dendrimers. Furthermore, synthesis of a hybrid dendrimer either by attaching small dendritic branches (dendrons) of different variety of chemical structures each with specific application to a single core molecule or by conjugating natural polymers to the dendrimers may also prove to be efficient and safe for future therapeutic applications.

Declaration of interest

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