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Review

Dendrimer toxicity: Let's meet the challenge

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ABSTRACT

Dendrimers are well-defined, versatile polymeric architecture with properties resembling biomolecules. Dendritic polymers emerged as outstanding carrier in modern medicine system because of its derivatisable branched architecture and flexibility in modifying it in numerous ways. Dendritic scaffold has been found to be suitable carrier for a variety of drugs including anticancer, anti-viral, anti-bacterial, antitubercular etc., with capacity to improve solubility and bioavailability of poorly soluble drugs. In spite of extensive applicability in pharmaceutical field, the use of dendrimers in biological system is constrained because of inherent toxicity associated with them. This toxicity is attributed to the interaction of surface cationic charge of dendrimers with negatively charged biological membranes *in vivo*. Interaction of dendrimers with biological membranes results in membrane disruption via nanohole formation, membrane thinning and erosion. Dendrimer toxicity in biological system is generally characterized by hemolytic toxicity, cytotoxicity and hematological toxicity. To minimize this toxicity two strategies have been utilized; first, designing and synthesis of biocompatible dendrimers; and second, masking of peripheral charge of dendrimers by surface engineering. Biocompatible dendrimers can be synthesized by employing biodegradable core and branching units or utilizing intermediates of various metabolic pathways. Dendrimer biocompatibility has been evaluated *in vitro* and *in vivo* for efficient presentation of biological performance. Surface engineering masks the cationic charge of dendrimer surface either by neutralization of charge, for example PEGylation, acetylation, carbohydrate and peptide conjugation; or by introducing negative charge such as half generation dendrimers. Neutral and negatively charged dendrimers do not interact with biological environment and hence are compatible for clinical applications as elucidated by various studies examined in this review. Chemical modification of the surface is an important strategy to overcome the toxicity problems associated with the dendrimers. The present review emphasizes on the approaches available to overcome the cationic toxicity inherently associated with the dendrimers.

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1. Introduction

Currently there is a growing interest in the use of polymers for delivery of therapeutic compounds. Polymer therapeutics constitutes an important class of drug delivery vehicles (Pignatello et al., 2009) such as nanoparticles, microspheres, polymeric micelles and dendrimers, which have proved their potential in drug and gene/DNA delivery. Dendrimers are emerging as promising drug delivery modules because of their well-defined nanoscale polymeric scaffold with low polydispersity index and controlled surface functionalities. These carriers share the features of defined compositions, high molecular mass and highly branched architecture with various names such as 'cascade molecules' or 'arborols'. These are the synthetic macromolecules with a tree-like structure first introduced by Tomalia et al. (1985) and Newkome et al. (1985). The term dendrimer arises from a Greek word '*dendron*' meaning 'tree/branch', due to its resemblance with a tree and '*meros*' meaning part. Dendrimers are defined as globular, monodisperse macromolecules with novel three-dimensional polymeric architectures in which all bonds emerge radially from a central focal point or core with repeat units that each contributes a branching point in dendrimer (Dykes et al., 2001; Frechet, 2002).

Dendrimers posses three distinguishable architectural components i.e. an interior core, interior layer (generations) composed of repeating units radially attached to the interior core, and exterior (terminal functionality) attached to outermost interior generation (Fig. 1). The higher generation dendrimers, due to their globular structure, occupy a smaller hydrodynamic volume compared to the corresponding linear polymers. The dendritic structure is characterized by layers between each generation (Boas and Heegaard, 2003; Jain and Gupta, 2008). The unique properties of dendrimers make them attractive carriers for drug and gene/DNA delivery (Zinselmeyer et al., 2002). Dendrimers have made an important contribution in fields of biomedicine, chemistry and biological science, material science, engineering and electronics.

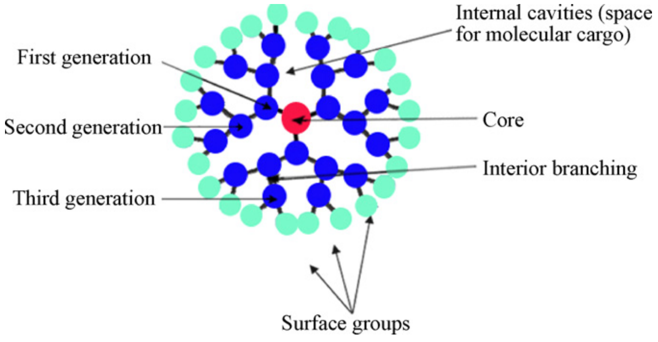

Fig. 1. General structure of dendrimers.

Dendrimers have proved their excellence as carrier for drugs and the bioactives because of their three-dimensional architecture, low polydispersity and high functionality. These enhance solubility (Jain and Gupta, 2008; Yiyun et al., 2005) and bioavailability of drugs (Emanuele et al., 2004; Chauhan et al., 2003) as well as offer the potential advantages of modifying the release of drug (Gajbhiye et al., 2009) including site-specific targeting (Bhadra et al., 2005). Dendrimers enhance solubility and bioavailability of poorly water-soluble drug by their container properties owing to their hydrophobic core and hydrophilic periphery (Jain and Gupta, 2008; Yiyun et al., 2005; Lim and Simanek, 2008). The possible mechanisms for dendrimer mediated solubility enhancement are hydrophobic interaction between drug and hydrophobic interior of dendrimer, hydrogen bonding and ionic interactions (Aulenta et al., 2003). Dendrimer mediated solubilization is affected by generation size, pH, core, temperature, polymeric architecture and functional groups (Gupta et al., 2007). Dendrimers may prolong the residence time of drug, increase the stability of bioactives, and protect it from biological environment. Methotrexate–dendrimer complex prepared in our laboratory was found to achieve pH-dependent release and significant targeting in the brain and bone marrow. This complex was also stable at physiological pH (Khopade and Jain, 1997). In addition to this dendrimers has also been used in gene therapy to introduce genetic material into target cells. Virus was the earliest gene delivery vector but its use is associated with high risk since they can induce strong immunogenic response in patient (Robbinsa and Ghivizzania, 1998). To circumvent this risk associated with virus many non-viral vectors were developed by scientists (Luo and Saltzman, 2000). Many polycationic polymers have been used in gene delivery including albumin, chitosan, poly-L-lysine (PLL), cationic liposomes, diethylaminoethyl dextran, polyethylenimine and dendrimers. Dendrimers such as polypropyleneimine (PPI) and polyamido amine (PAMAM) (Figs. 2 and 3), with positive charge on their surface, have the ability for condensation of DNA followed by transfection (Dufes et al., 2005; Omidi et al., 2005). Dendrimers form a stable complex with DNA that can penetrate cell membrane due to its positive charge to release DNA inside cells. They can be

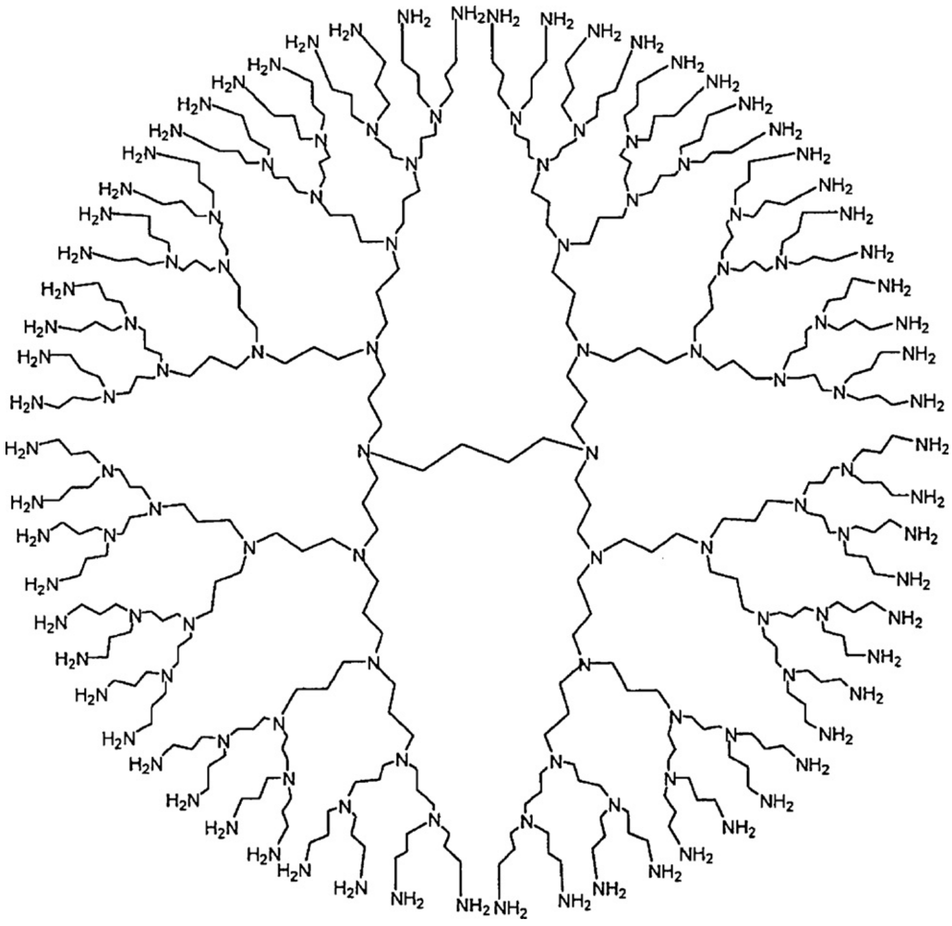

Fig. 2. PPI dendrimer.

surface modified to enhance transfection efficiency and specificity to target cells (Li et al., 2007). Dendrimers offer a better opportunity for anticancer drug delivery. Various nanocarriers have been used for the delivery of anticancer agents (Amiji, 2006) but dendrimer has proved itself a resourceful moiety in the therapy of malignant growths because of its vital applicability in the delivery of antineoplastic drug (Wu et al., 1994), contrast agents (Konda et al., 2001; Backer et al., 2005) and in gene therapy, including its pivotal role in neutron capture therapy (Shukla et al., 2003; Nishiyama et al., 2009), photodynamic therapy (Hu et al., 2008), and photothermal therapy (Agrawal et al., 2008). Apart from these, dendrimers are also useful as catalyst (Nlate et al., 2006) and organic light-emitting diodes (Brauge et al., 2006). Nlate et al. (2006) synthesized 9- and 27-armed tetrakis (diperoxotungsto) phosphate-cored dendrimers to be used as a recoverable and reusable catalyst with hydrogen peroxide in the oxidation of alkenes, sulfides and alcohols. Phosphorus dendrimers bearing fluorescent end groups such as naphthalene, anthracene and pyrene were also synthesized for the embellishment of organic light-emitting diodes (Brauge et al., 2006).

2. Dendrimers toxicity

Unarguably dendrimers have brought the tremendous advances in the field of drug delivery. A great optimism is expected regarding their potential in biomedical field but because of nanometric size i.e. 1–100 nm they may interact effectively and specifically with the components of cell such as plasma membranes, cell organelles (endosomes, mitochondria, nucleus) and proteins such as enzyme etc., as all these cellular components are themselves nanometer in size range (Lee et al., 2008; Choi et al., 2006). Most types of nanoparticles developed by scientists are non-selective. Nanoscale size range of nanoparticles made them to interact with or permeate through plasma membrane of biological system. These aspects of nanocarriers are of substantial interest in cell transfection strategy and are being developed for *in vivo* gene delivery (Lee et al., 2008; Choi et al., 2006; Wang et al., 2007, 2009). But there is another aspect of non-selective uptake of these nanoparticles i.e. their potential to cause cytotoxicity. In the following section we will focus on the cytotoxic behavior of dendrimers, the mechanism by which these cationic macromolecules induce cytotoxicity and possible strategies to alleviate dendrimer toxicity.

Regardless of the extensive pharmaceutical and biomedical applications of dendrimers, toxicity associated due to terminal-NH_2 groups and multiple cationic charge, limits their candidatures for clinical applications (Malik et al., 2000; Wilbur et al., 1998; Brazeau et al., 1998). Dendrimers like PPI, PAMAM and PLL exert significant *in vitro* cytotoxicity due to their surface cationic groups (Agashe et al., 2006; Kolhatkar et al., 2007). Evidence regarding dendrimer safety is conflicting (Roberts et al., 1996). There are reports of concentration and generation-dependent toxicity of free amine groups present at their periphery (Chen et al., 2004). Chen et al. (2004) reported the cytotoxicity of cationic melamine dendrimers having surface groups like amine, guanidine, carboxylate, sulphonate or phosphonate and concluded that cationic dendrimers were much more cytotoxic than anionic or PEGylated dendrimers. Not only dendrimers but also cationic macromolecules in general cause destabilization of the cell membrane and result in cell lysis (Rittner et al., 2002; Hong et al., 2006a; Fischer et al., 2003). This is an important finding and a possible tip to scientists working in the relevant areas of research.

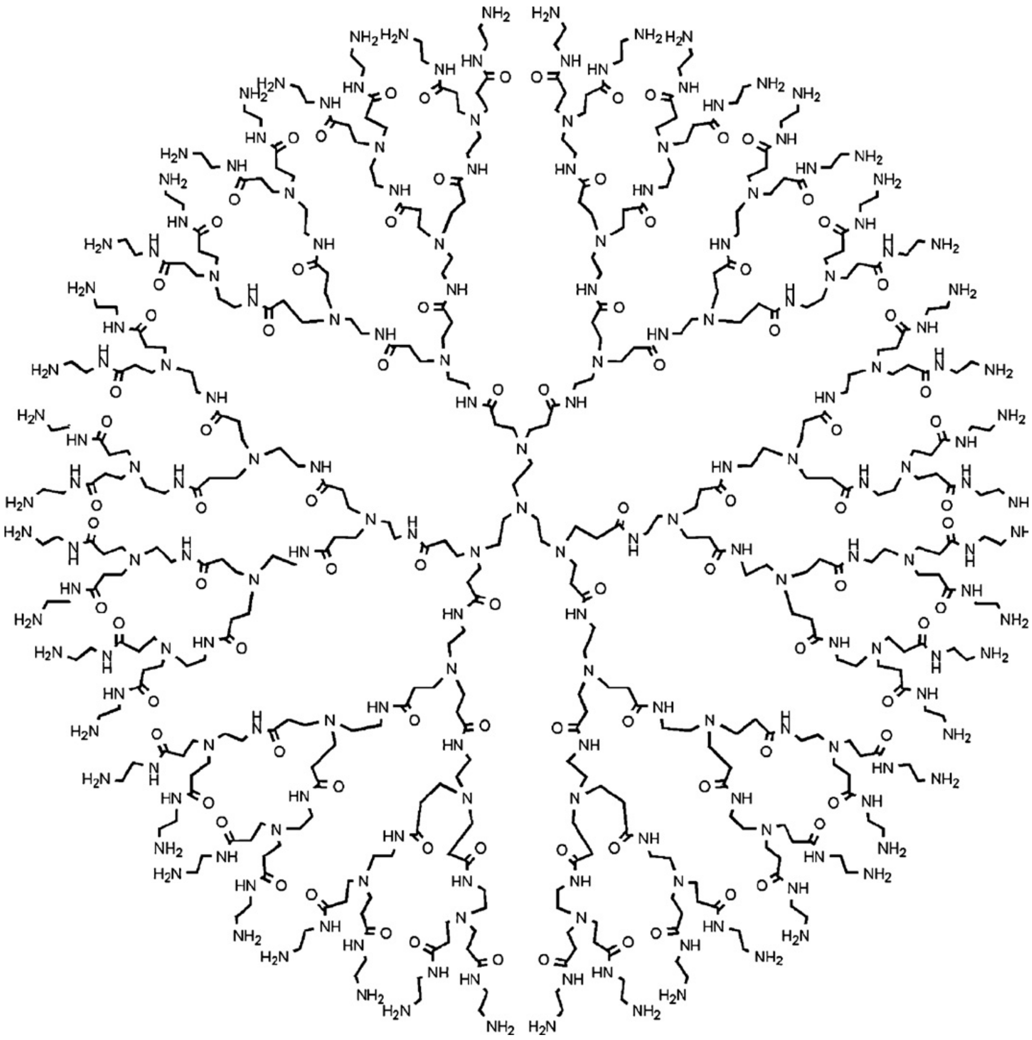

Fig. 3. PAMAM dendrimer.

2.1. Cytotoxicity

Jevprasesphant et al. (2003) investigated cytotoxicity of PAMAM dendrimers in Caco-2 cell lines and observed a significant cytotoxicity with these dendrimers. Malik et al. (2000) also reported the cytotoxicity of PPI and PAMAM dendrimers in three cancer cell lines B16F10, CCRF and HepG2. The study was a breakthrough in this direction. Agashe et al. (2006) studied the cytotoxicity of plain 5.0 G PPI, amino acid protected and carbohydrate-coated PPI dendrimers in HepG2 and COS-7 cell lines and observed the effect of terminal functional groups, concentration and incubation time on cytotoxicity. For 5.0 G PPI cell viability was studied using different concentration of dendrimers over incubation times of 24, 48 and 72 h (Table 1). Cell viability was found to decrease with increase in concentration and incubation time. The cytotoxicity was found to be concentration- as well as time-dependent for 5.0 G PPI and attributed to the presence of free primary amine groups in 5.0 G PPI and the positive charge associated with them. COS-7 cell lines showed higher cell viability as compared with HepG2 at all concentration and incubation time points.

Stasko et al. (2007) evaluated cytotoxicity and membrane disruption by PPI dendrimers, PEG conjugated PPI dendrimers and PPI dendrimers with peripheral neutral acetamide groups, on cultured human umbilical vein endothelial cells (HUVEC) and found that the plain PPI dendrimers demonstrated drastic time-dependent changes in the plasma membrane permeability and prominent cytotoxicity (Table 2).

Table 1
Cytotoxicity of 5.0 G PPI dendrimer against HepG2 and COS-7 cell lines (Agashe et al., 2006).

Conc. of 5.0 G PPI dendrimer (mg/ml)	Cell lines	Time of incubation (h)	Cell viability (%)
0.001	HepG2	24	66.8
0.001	COS-7	24	71.5
0.001	HepG2	72	16.8
0.001	COS-7	72	23.1
1.0	HepG2	24	7.6
1.0	COS-7	24	11.7
1.0	HepG2	72	1.7
1.0	COS-7	72	2.3

Table 2
Cytotoxicity studies on PPI and PAMAM dendrimers.

Type of dendrimers	Cell lines	Observations	References
5.0 G PPI dendrimers	a. Hep G2 cells b. COS-7 cells	a. Cell viability 66.8% at 0.001 mg/ml and 7.6% at 1 mg/ml conc. after 24 h b. Cell viability 71.5% at 0.001 mg/ml and 11.7% at 1 mg/ml conc. after 24 h	Agashe et al. (2006)
	Cultured human umbilical vein endothelial cells (HUVEC)	Drastic time-dependent changes in the plasma membrane permeability and prominent cytotoxicity	Stasko et al. (2007)
Cationic or whole generation PAMAM dendrimers a. 2.0 G b. 3.0 G, 4.0 G	Caco-2 cells	a. Cytotoxic at concentration above 700 μM b. Cytotoxic at all concentrations examined by investigator	Jevprasesphant et al. (2003)

2.2. Hemolytic toxicity

The free cationic terminal groups of dendrimers interact with RBCs and this polycationic nature of dendrimers leads to hemolysis (Table 3) (Bhadra et al., 2003; Asthana et al., 2005). The hemolytic toxicity of dendrimers was investigated by mixing dendrimers and RBC suspension followed by incubation for definite time intervals at 37 °C, centrifugation at 3000 rpm for 15 min followed by analysis of the supernatant at 540 nm spectrophotometrically against blank (in normal saline), and percentage hemolysis was calculated against absorbance factor of 100% hemolytic sample in distilled water (Agashe et al., 2006; Agrawal et al., 2007).

Bhadra et al. (2003) developed 4.0 G PAMAM dendrimers for delivery of an anticancer drug 5-FU and found its haemolytic toxicity to be ~15.3–17.3%. Later Asthana et al. (2005) also evaluated these dendrimers for hemolytic toxicity and observed up to 18% hemolytic toxicity, close to the value reported by Bhadra et al. (2003). Malik et al. (2000) reported 3.0 G PPI and PAMAM dendrimers to induce hemolysis above a concentration of 1 mg/ml. Bhadra et al. (2005) observed marked hemolysis with 4.0 and 5.0 G PPI dendrimers. The authors found 35.7% hemolysis with 4.0 G PPI dendrimers and 49.2% hemolysis with 5.0 G PPI dendrimers. Agashe et al. (2006) also investigated the hemolytic toxicity of 5.0 G PPI dendrimers. They observed 34.2 ± 0.2%, 51.6 ± 0.3% and 86.2 ± 0.6% hemolysis with 5.0 G PPI dendrimers at concentration of 1 mg/ml after incubation for 1, 2 and 4 h, respectively. PLL dendrimers were also found to induce hemolysis *in vitro* (Agrawal et al., 2007). Agrawal et al. (2007) found 14.1 ± 1.02% hemolysis with 4.0 G PLL dendrimers. Available reports suggest that higher generation dendrimers may have greater hemolytic toxicity, which may be ascribed to the greater overall cationic charge.

2.3. Haematological toxicity

Dendrimers induce hemolysis owing to interaction with RBCs and they also influence hematological parameters attributable to polycationic nature of the uncoated dendrimers. The effect of PPI dendrimers on different blood parameters including white blood corpuscles (WBCs), red blood corpuscles (RBCs), haemoglobin (Hb), haematocrit (HCT) and mean corpuscular haemoglobin (MCH) was determined using Erma particle counter by Agashe et al. (2006). Authors observed a significant decrease in RBC count, a substantial increase in WBC count, decrease in Hb content and MCH value, and a considerable difference in HCT value between control and PPI dendrimers. RBCs count, Hb, HCT and MCH values were found to decrease drastically to 4.43 ± 1.15, 8.26 ± 1.39, 27.06 ± 3.16 and 18.82 ± 1.06 from normal values of 7.15 ± 1.37, 14.89 ± 1.35, 41.69 ± 5.51 and 20.76 ± 1.35, respectively with 5.0 G plain PPI dendrimers (Table 4).

Bhadra et al. (2005) compared the effect of primaquine phosphate loaded dendrimers on various haematological parameters in male albino rats (Sprague–Dawley strain) and found RBC count to decrease below normal values by $1.1 \pm 0.05 \times 10^6$ RBCs/μl and WBC count to increase by $2.7 \pm 0.3 \times 10^3$ μl^{-1} cells as compared to normal values by uncoated PPI dendrimers. Later Agrawal et al. (2007) also observed a significant increase in WBCs count and decrease in RBCs count, with 4.0 G PLL dendrimers (Table 4). The RBCs count was found to decrease up to $7.5 \pm 0.3 \times 10^6$ μl^{-1} and WBCs count was found to increase up to $15.2 \pm 0.4 \times 10^6$ μl^{-1} with 4.0 G PLL dendrimers. From these studies we may conclude that the cationic dendrimers might exhibit significant impairment in hematological parameters i.e. a considerable increase in WBCs count, decrease in RBCs count, Hb, HCT and MCH values and this underlines the need for a strategy to render them more biocompatible.

2.4. Immunogenicity

Some scientists investigated the immunogenicity of dendrimers and concluded that dendrimers shows no or only weak immunogenicity (Roberts et al., 1996; Agashe et al., 2006; Rajananthanan et al., 1999). Roberts et al. (1996) inspected the immunogenicity of PAMAM dendrimers by immunoprecipitation and Ouchterlony double-diffusion assay but observed no signs of immunogenicity with the dose range of 0.1–0.0001 μM. Agashe et al. (2006) investigated the immunogenicity of 5.0 G PPI dendrimers in Balb/C mice using ELISA for monitoring antibody titre and reported that dendrimers was unable to provoke any detectable humoral immune response under the experimental conditions. This indicates that

Table 3
Hemolytic toxicity of some dendrimers.

Type of dendrimers	Percent hemolysis	References
5.0 G PPI dendrimers	86.2 ± 0.6% (at 1 mg/ml conc. after incubation of 4 h) 49.2%	Agashe et al. (2006) Bhadra et al. (2005)
4.0 G PAMAM dendrimer	18% ~15.3–17.3%	Asthana et al. (2005) Bhadra et al. (2003)
3.0 G PAMAM dendrimers	Hemolytic above a concentration of 1 mg/ml	Malik et al. (2000)
3.0 G PPI dendrimers	Hemolytic above a concentration of 1 mg/ml	Malik et al. (2000)
4.0 G Poly-L-lysine Dendrimers	14.1 ± 1.02%	Agrawal et al. (2007)
4.0 G PPI dendrimers	35.7%	Bhadra et al. (2005)

Table 4
Effect of uncoated and functionalized dendrimers on hematological parameters.

S. No.		Type of dendrimers	Hematological parameters					References
			RBCs	WBCs	Hb	HCT	MCH	
A.	1.	5.0 G PPI dendrimers	4.43 ± 1.15	12.01 ± 1.23	8.26 ± 1.39	27.06 ± 3.16	18.82 ± 1.06	Agashe et al. (2006)
	2.	Glycine-coated 5.0 G PPI dendrimers	7.01 ± 1.41	9.01 ± 1.02	12.76 ± 1.31	38.71 ± 3.18	18.15 ± 0.78	
	3	Phenylalanine-coated 5.0 G PPI dendrimers	6.99 ± 1.22	8.99 ± 1.36	12.51 ± 1.35	38.38 ± 3.14	18.01 ± 1.08	
	4.	Mannose-coated 5.0 G PPI dendrimers	7.36 ± 1.01	8.24 ± 1.89	13.46 ± 1.78	41.82 ± 4.32	18.41 ± 0.53	
	5.	Lactose-coated 5.0 G PPI dendrimers	7.24 ± 1.24	8.37 ± 1.46	13.01 ± 1.59	41.02 ± 4.62	18.15 ± 1.21	
	6.	Control	7.15 ± 1.37	8.25 ± 1.67	14.89 ± 1.35	41.69 ± 5.51	20.76 ± 1.35	
B.	1.	4.0 G poly-L-lysine dendrimers	7.5 ± 0.3	15.2 ± 0.4	np	np	np	Agrawal et al. (2007)
	2.	Galactose coated 4.0 G poly-L-lysine dendrimers	8.8 ± 0.2	11.3 ± 0.4	np	np	np	
	3.	Control	9.1 ± 0.3	10.8 ± 0.6	np	np	np	

RBCs = red blood corpuscles; WBCs = white blood corpuscles; Hb = hemoglobin; HCT = hematocrit; MCH = mean corpuscular hemoglobin; np = not performed.

dendrimers are treated by the host immune system as ‘native’ and this is a positive sign for its proposed drug delivery caliber. However paucity of available reports warrants corroboration by unequivocal data to arrive at any meaningful conclusion.

2.5. *In vivo toxicity*

In vivo toxicity studies are essential to prove the safety of any drug delivery studies. Few scientists have performed systematic study on *in vivo* toxicity of dendrimers. Roberts et al. (1996) studied *in vivo* toxicity of 3.0, 5.0 and 7.0 G PAMAM dendrimers in Swiss–Webster mice wherein only 7.0 G PAMAM dendrimers were observed to produce potential biological complications. Authors finally concluded that dendrimers did not exhibit properties that would preclude their use in biological applications. Later Rajananthanan et al. (1999) studied the immunopotential properties of novel molecular aggregate formulation based on 5.0 G dendrimeric polymer and found this formulation to be non-toxic to mice and to produce antigen-specific antibody response. Malik et al. (2000) studied the *in vivo* biodistribution of ^{125}I-labelled PAMAM dendrimers to determine the toxicological profile of this polymeric carrier. In this study, authors observed that after i.p. and i.v. administration cationic PAMAM dendrimers were rapidly cleared from circulation while only 0.1–1.0% of the dose was recovered in blood 1 h post administration. A detailed study on *in vivo* behavior of melamine dendrimers was performed by Neerman et al. (2004). In acute toxicity studies they administered 2.5, 10, 40 and 160 mg/kg of melamine dendrimers in mice by i.p. injection and observed 100% mortality in 6–12 h post injection of dose at 160 mg/kg. Hepatotoxicity was observed at 40 mg/kg as indicated by increase in liver enzyme activity. Neither mortality nor renal damage was observed at subchronic doses of 2.5 and 10 mg/kg. From the aforesaid account it may be concluded that the administration of dendrimers to biological system needs a detailed study on their *in vivo* disposition, which depends on their structural components core, interior generations and particularly surface groups.

3. Membrane interaction

The rapidly developing field of nanotechnology is apprehended to emerge out as another source of potential toxicity to human health through inhalation, skin uptake, and injection of engineered nanomaterials. Toxicity of nanocarriers involves physiological, physiochemical and molecular consideration. Regardless of potential application in diagnosis, imaging and therapy there are also controversy generated by the possible toxic health effects of nanoparticles and this has raised the urgent need for evaluation of safety and potential hazards of engineered nanostructures and nanodevices (Hoet et al., 2004).

Tajarobi et al. (2001) determined permeability of PAMAM dendrimers (0.0–4.0 G) across Madin–Darby Canine Kidney (MDCK) cell lines and investigated the influence of physiochemical parameters like size, molecular weight, molecular geometry, and number of surface amine groups of PAMAM dendrimers on their permeability across Caco-2 cell monolayers. Authors found that the permeability of the PAMAM dendrimers increased in the following order $4.0\,\mathrm{G} > > 1.0\,\mathrm{G} \approx 0.0\,\mathrm{G} > 3.0\,\mathrm{G} > 2.0\,\mathrm{G}$ across MDCK cell lines. It was concluded that polymer size and modulation of cell membrane by cationic dendrimers played an important role in the transepithelial transport of dendrimers (Tajarobi et al., 2001). El-Sayed et al. (2002) studied the influence of PAMAM dendrimers on integrity, paracellular permeability, and viability of Caco-2 cell monolayers by measuring the transepithelial electrical resistance (TEER), mannitol permeability, and leakage of lactate dehydrogenase (LDH) enzyme, respectively and observed increase in LDH leakage with increase in concentration of dendrimers, incubation time and generation. Permeability of polyamine-conjugated dendrimers across Caco-2 cell monolayers was also monitored by measuring decrease in TEER with both, the PAMAM- and the polyamine-conjugated dendrimers. In this study, Pisal et al. (2008) conjugated amine terminals of 4.0 G PAMAM dendrimers with polyamines, arginine and ornithine, and suggested that paracellular transport is one of the transport mechanisms across the epithelial membranes for both modified and unmodified dendrimers. Lee and Larson (2008) performed molecular dynamics (MD) simulations of multiple copies of PLL and PAMAM dendrimers in DMPC bilayers with explicit water using coarse-grained model and concluded (i) firstly, membrane disruption is enhanced at higher concentrations and charge densities of both spheroidally shaped dendrimers and linear PLL polymers, (ii) secondly, larger molecular size enhances membrane disruption and pore formation only for dendrimers but not for the linear PLL polymers. These results indicated that a relatively rigid spheroidal shape may be more efficient than a flexible linear shape in increasing membrane permeability. Dendrimers thus interact with biological membrane to increase permeability but this increase in permeability also has a negative aspect i.e. disruption of biological membrane, which finally leads to toxicity of these carriers to biological environment.

Many scientists have studied the interaction of dendrimers and other nanocarriers with biological membrane and described that cationic macromolecules interact with negatively charged biological membrane to produce nanoscale holes and cell lysis. These studies have been performed on both, model biological membranes as well as living cell membrane models (Fischer et al., 2003; Hong et al., 2006b, 2004; Mecke et al., 2004, 2005a,b,c; Leroueil et al., 2008; Zhang and Smith, 2000; Ottaviani et al., 1998; Manunta et al., 2004, 2006).

3.1. *Interaction with lipid bilayers*

Interaction of polycationic dendrimers and similar cationic polymers with biological membranes has been investigated employing an assortment of analytical techniques (Hong et al., 2006b, 2004; Mecke et al., 2004, 2005a; Leroueil et al., 2008; Zhang and Smith, 2000). Interaction with these drug carriers increases the permeability and decreases the integrity of biological membrane. This leads to the leakage of cytosolic proteins such as lactate dehydrogenase (LDH) and luciferase (Luc.) etc, and finally membrane disruption and cell lysis (Mecke et al., 2005b,c).

Ottaviani et al. (1998) investigated the interaction of PAMAM dendrimers with liposomes formed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and suggested that dendrimers could be exploited as vital carrier for gene/DNA and other biomolecules. Authors investigated the interaction between dendrimers and liposomes by negative staining transmission electron microscopy, dynamic light scattering (DLS), and electron paramagnetic resonance (EPR). In the analysis of EPR spectra authors found that at temperatures below the critical temperature transition of DMPC from liquid-crystal phase to the gel (or solid) phase, the vesicle structure changed and became more rigid and organized whereas it is generally assumed that the area of the phosphatidylcholine head group remains almost unchanged upon transition. Based on this fact the authors assumed that predominantly dipolar and electrostatic interactions occurred between dendrimer and liposome surface groups. From the changes in parameter obtained from electron spin-echo envelope modulation (ESEEM) and continuous wave-electron paramagnetic resonance the authors concluded that dendrimer–vesicle interactions are stronger for higher generations than for lower generations. Finally they suggested that the dendrimers interacted with the membrane surface, but did not permanently perturb the membrane properties (enhanced biocompatibility); measurements at low temperatures indicated that interaction was more effective for protonated dendrimers at larger sizes (higher generations), and therefore they have more potential as carriers of biomolecules to approach the cell membrane. In continuation Zhang and Smith (2000) evaluated disruption properties of 4.0–7.0 G PAMAM dendrimers and found that 6.0 and 7.0 G dendrimers were effective at inducing leaky fusion of anionic, large unilamellar vesicles. Authors interpreted the enhanced membrane disruption in terms of a membrane bending model, which is based on the hypothesis that a rigid, polycationic dendrimer sphere uses electrostatic forces to bend a malleable, anionic membrane and induces bilayer packing stresses. This bending model is biomimetic since the protein-induced membrane bending is currently thought to be an important factor in the fusion mechanism of influenza virus.

Later Hong et al. (2004) and Mecke et al. (2004) explored the mechanism by which cationic PAMAM dendrimer induces membrane damage. Authors used DMPC lipid bilayers for probing the mechanism of cationic PAMAM dendrimer-induced membrane damage. Similar studies were also performed with some nanoparticles to investigate the interaction of other polycationic polymers including poly-L-lysine (PLL), polyethyleneimine (PEI) and diethylaminoethyl-dextran (DEAE-DEX), which are established as imperative carrier for gene and drug delivery (Hong et al., 2006b). Authors observed that 7.0 G PAMAM dendrimers instigated the nanoscale holes of diameter between 15 and 40 nm in lipid bilayers whereas 5.0 G PAMAM dendrimers did not induce nanoscale hole formation but expanded the size of pre-existing defects in the bilayers. It was hypothesized that these holes are upshots of electrostatic interactions between dendrimers and supported lipid bilayers and the formation of dendrimer-nucleated lipid vesicles (Mecke et al., 2004).

Leroueil et al. (2008) employed atomic force microscopy to determine the common mechanism of interaction of nanoparticles with lipids and physical disruption of lipid membranes. Authors performed the experiments with variety of nanoparticles including cell penetrating peptide (MSI-78), protein (TAT), dendrimers (PAMAM and pentanol-core PAMAM dendrons), polycationic polymers (polyethyleneimine and diethylaminoethyl-dextran), and with inorganic particles (Au–NH_2, SiO_2–NH_2) on supported lipid bilayers. They found that these polycationic nanoparticles interacted with biological membranes and induce cell disruption by formation of nanoholes, membrane thinning, and/or membrane erosion mainly.

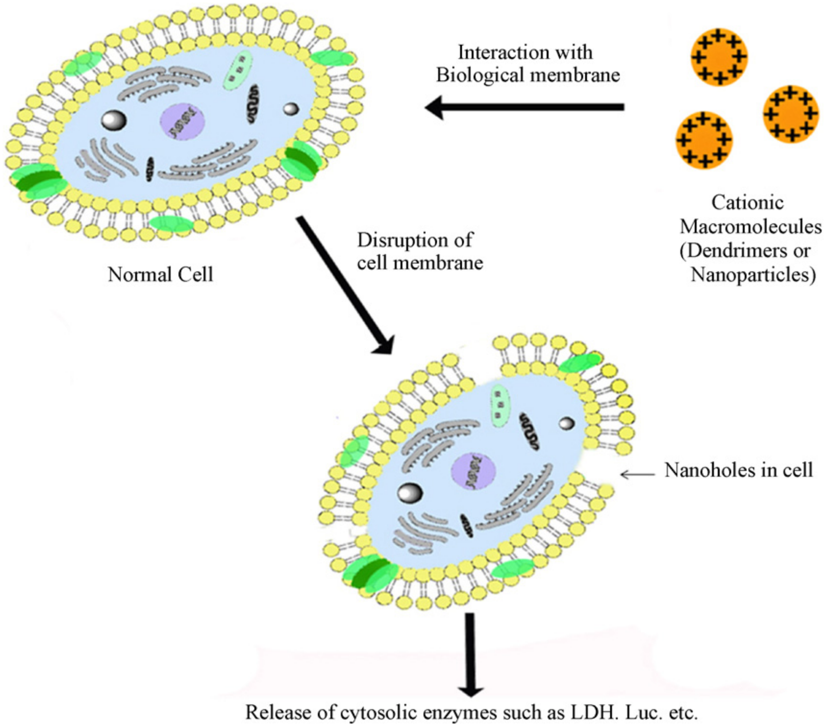

Fig. 4. Mechanism of interaction of cationic macromolecules with biological membrane.

Conclusively, dendrimers with surface cationic charges interact with negatively charged biological membrane because of their positive charge. This interaction leads to membrane disruption by inducing formation of nanoholes or causing membrane thinning or erosion that is responsible for the toxicity of dendrimers. Ultimately this leads to leakage of cytosolic enzymes, and cell lysis (Fig. 4).

3.2. Interaction with cell membrane

Available literatures widely suggest a concluding trend that cationic macromolecules show a considerable cytotoxicity. Fischer et al. (2003) performed a comparative *in vitro* cytotoxicity study of different cationic gene delivery systems on L929 mouse fibroblasts cells using MTT assay and measuring the release of the cytosolic enzyme lactate dehydrogenase (LDH). The study was performed on following cationic macromolecules: poly(ethylenimine) (PEI), poly(L-lysine), poly(diallyl-dimethyl-ammonium chloride), diethylaminoethyl-dextran, poly(vinyl pyridinium bromide), starburst dendrimer, cationized albumin and native albumin. Authors found the following ranking of polymers with regard to cytotoxicity with native albumin being the least toxic followed by cationized albumin: starburst dendrimer, poly(vinyl pyridinium bromide), diethylaminoethyl-dextran, poly(diallyl-dimethyl-ammonium chloride); PEI and PLL being the most cytotoxic. They also studied the nature of cell death induced by these carriers and assumed that cell lysis was not caused by apoptosis (necrotic cell reaction) since it was found that cell nuclei retained its size and chromatin was homogeneously distributed but cell membranes lost their integrity very rapidly at an early stage. Manunta et al. (2004) investigated the uptake procedure of dendrimer–DNA complex (dendriplexes) in endothelial cell line. Authors also assessed whether the internalization of dendriplexes took place randomly on the cell surface or at preferential sites such as membrane rafts. They observed that membrane rafts were important for the internalization of non-viral vectors in gene therapy; dendrimer conducts gene delivery via cholesterol dependent pathway (Manunta et al., 2004). Later Manunta et al. (2006) investigated the internalization and transfection properties of dendriplexes using HepG2 and HeLa cell lines that express few caveolae and observed that dendrimer–DNA complex may use different internalization pathways in different cells but enhanced in the presence of caveolin into HeLa, HepG2 and endothelial cells.

Hong et al. (2006b, 2004) also examined the disruption events on living cells *in vitro* using KB and Rat2 cell membranes through atomic force microscopy (AFM), enzyme assays, flow cell cytometry and fluorescence microscopy. They found a dose-dependent release of the cytoplasmic proteins lactate dehydrogenase (LDH) and luciferase (Luc), which indicated that interaction of dendrimers with membrane resulted in decreased integrity of cell membrane and lead to induction of permeability with subsequent internalization of dendrimers. Up to a concentration of 500 nm neither 5.0 G amine- nor acetamide-terminated PAMAM dendrimers were cytotoxic. These researchers also realized that the induction of permeability by dendrimers was not permanent and leaking of cytoplasmic proteins returned to normal levels upon removal of dendrimers. 5.0 G dendrimers modified with acetamide do not internalize to the cells and hence internalization is attributable to interaction between cationic macromolecules and cell membrane followed by increase in permeability, which is pivotal for the delivery of biomolecules inside the cell.

4. Solutions for toxicity issues

There is a great need to devise methods to circumvent the cytotoxicity and hemolytic toxicity associated with these promising drug delivery systems so that they can be made biomimetic (Tables 5 and 6). Various strategies have been proposed by different scientists to alleviate the toxicity associated with dendrimers (Fig. 5). In this section we will examine the strategies which may be employed to resolve the issues encountered in the use of parent dendrimers as drug delivery vehicles.

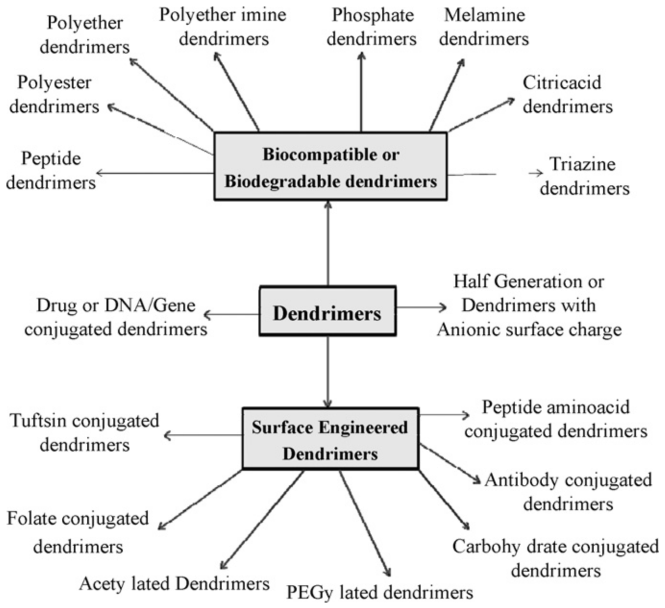

Fig. 5. Various strategies to alleviate toxicity associated with dendrimers.

4.1. Biodegradable/biocompatible dendrimers

The first approach for allaying the toxicity of dendrimers involves development of some less toxic dendrimers having biodegradable core and branching units as reported by various researchers. Many scientists have employed monomers in the synthesis of dendrimers which are metabolic products of various biological pathways and hence they lead to the synthesis of biodegradable dendrimers (Agrawal et al., 2007). Biodegradable/biocompatible carrier is a dream in the development of a drug delivery system that can deliver the drug at a rate dictated by the needs of the body over a specified period of treatment. So these dendrimers may fulfill the dream of the pharmaceutical scientists to develop an ideal carrier system for therapeutic agents. Relevant account of such type of dendrimers is presented below.

4.1.1. Polyether dendrimers

Polyether dendrimers (Fig. 6) were developed by Hawker and Frechet (1990) by convergent route using 1,1,1-tris(4′-hydroxyphenyl) ethane as the core material and benzylic bromide and 3,5-dihydroxybenzyl alcohol as branching material to form dendrons. Later, aliphatic polyether dendrimers were also synthesized by convergent route (Jayaraman and Frechet, 1998). Saville et al. (1995) elucidated the structure of polyether dendrimers by neutron reflectivity and IIA isotherm measurement in the air water interface and observed a collapse of fourth generation monodendron to a bilayers structure, ellipsoidal molecules in the layer next to the water interface due to compression and spherical molecule having a diameter of 21 Å in the layer next to air from neutron reflectivity measurements. Leon and Frechet (1995) analyzed aromatic polyether dendrimers with differing chain end functionalities and molecular topologies by matrix-assisted laser desorption ionization (MALDI-TOF) mass spectrometry. In preliminary studies with polyether dendrimers Malik et al. (2000) found that den-

Table 5
Cytotoxicity studies on biocompatible and surface engineered dendrimers.

Type of dendrimers	Cell lines	Results	References
Polyether imine dendrimers	a. Human breast cancer T47D b. African green monkey CV-1	Cell survival >98% up to 100 mg/ml of dendrimer solution	Krishna et al. (2005)
SN-38 complexed G4- PAMAM dendrimers	Caco-2 cells	No appreciable toxicity (cell viability >90% after incubation for 2 h at 0.1 μM conc.)	Kolhatkar et al. (2008)
Glycine-coated 5.0 G PPI dendrimers	a. Hep G2 cells b. COS-7 cells	a. Cell viability 95.37% at 1 mg/ml conc. after 24 h b. Cell viability 96.1% at 1 mg/ml conc. after 24 h	Agashe et al. (2006)
Phenylalanine-coated 5.0 G PPI dendrimers	a. Hep G2 cells b. COS-7 cells	a. Cell viability 96.2% at 1 mg/ml conc. after 24 h b. Cell viability 93.0% at 1 mg/ml conc. after 24 h	Agashe et al. (2006)
Mannose-coated 5.0 G PPI dendrimers	a. Hep G2 cells b. COS-7 cells	a. Cell viability 95.2% at 1 mg/ml conc. after 24 h b. Cell viability 98.1% at 1 mg/ml conc. after 24 h	Agashe et al. (2006)
Lactose-coated 5.0 G PPI dendrimers	a. Hep G2 cells b. COS-7 cells	a. Cell viability 94.7% at 1 mg/ml conc. after 24 h b. Cell viability 97.71% at 1 mg/ml conc. after 24 h	Agashe et al. (2006)
5.0 G PPI dendrimers with peripheral neutral acetamide groups	Cultured human umbilical vein endothelial cells (HUVEC)	Decrease in cytotoxicity was observed	Stasko et al. (2007)
Carboxylic acid-terminated PPI dendrimers	B16F10 cells	Not cytotoxic up to concentration of 1 mg/ml after 24 h	Malik et al. (2000)
PEGylated PPI dendrimers	Cultured human umbilical vein endothelial cells (HUVEC)	Elimination or reduction of acute cytotoxicity of plain PPI dendrimers	Stasko et al. (2007)
Carboxylic acid-terminated PAMAM dendrimers (Half generation)	B16F10 cells	Not cytotoxic up to concentration of 1 mg/ml after 24 h	Malik et al. (2000)
Anionic or half generation PAMAM dendrimers (2.5 G, 3.5 G)	Caco-2 cells	No cytotoxicity up to 1 Mm concentration.	Jevprasesphant et al. (2003)
Lauroyl- and PEGylated PAMAM dendrimers	Caco-2 cells	Seven fold reduction in cytotoxicity compared to unmodified dendrimers	Jevprasesphant et al. (2003)

drimers with carboxylate and malonate surface groups were not lytic at 1 h but after incubation for 24 h they were lytic (Malik et al., 2000).

4.1.2. *Polyester dendritic system*

Bo et al. (1997) furnished a method for the rapid synthesis of polyester dendrimers using methyl 3,5-dihydroxybenzoate and 3,5-dibenzyloxybenzoic acid as monomers (Fig. 7). Ihre et al. (2001, 2002) designed and synthesized dendritic polyester system to improve tumor targeting and therapeutic efficacy due to the EPR effect observed in tumor tissue. These systems were based on the monomer unit 2,2-bis(hydroxymethyl)propanoic acid (Ihre et al., 2001, 2002). Galie et al. (2006) employed these dendrimers for boron neutron capture therapy containing carborane. Hirayama et al. (2005) synthesized new aliphatic polyester dendrimers from benzyl acetoacetate and t-butyl acrylate by acetoacetic acid ester synthesis using divergent growth method. Goh et al. (2002) proposed a new approach for the controlled synthesis of multicomponent dendrimers wherein they synthesized three oligonucleotide–dendron conjugates using solid phase techniques and hybridized to create a second generation polyester dendrimer with DNA as a core and bearing two types of peripheral functional groups. Another example of polyester dendrimers is polyglycerol succinic acid dendrimers (Luman et al., 2003), synthesized by convergent synthesis from biocompatible or biodegradable building blocks including succinic acid, glycerol and PEG. Hirayama et al. (2005) synthesized new aliphatic polyester dendrimers from benzyl acetoacetate and t-butyl acrylate by acetoacetic acid ester synthesis using divergent growth method. Gillies et al. (2005) investigated the biological performance of these dendrimers and found that the polyester dendrimer–poly(ethylene oxide) hybrids were non-toxic to cells and high levels of tumor accumulation were found in mice bearing subcutaneous B16F10 tumor.

Table 6
Hemolytic toxicity profile of surface modified dendrimers.

Type of dendrimers	% Hemolysis	References
Glycine-coated 5.0 G PPI dendrimers	4.9 ± 0.2% (at 1 mg/ml conc. after incubation of 4 h)	Agashe et al. (2006)
Phenylalanine-coated 5.0 G PPI dendrimers	3.3 ± 0.4% (at 1 mg/ml conc. after incubation of 4 h)	Agashe et al. (2006)
Mannose-coated 5.0 G PPI dendrimers	2.9 ± 0.6% (at 1 mg/ml conc. after incubation of 4 h)	Agashe et al. (2006)
Lactose-coated 5.0 G PPI dendrimers	2.2 ± 0.3% (at 1 mg/ml conc. after incubation of 4 h)	Agashe et al. (2006)
Half generations of carboxylic acid-terminated PAMAM dendrimers	Negligible hemolytic toxicity	Bhadra et al. (2003)
PEGylated PAMAM dendrimers	<5%	Bhadra et al. (2003)
Carboxylic acid-terminated PAMAM dendrimers (Half generation)	Not hemolytic up to concentration of 2 mg/ml	Malik et al. (2000)
Carboxylic acid-terminated PPI dendrimers	Not hemolytic up to concentration of 2 mg/ml	Malik et al. (2000)
Oligoethyleneoxide-terminated carbosilane dendrimers	Not hemolytic up to concentration of 2 mg/ml	Malik et al. (2000)
Galactose coated 4.0 G poly-L-lysine dendrimers	7.3 ± 2.8%	Agrawal et al. (2007)
Galactose coated 4.0 G PPI dendrimers	7.1%	Bhadra et al. (2005)
Galactose coated 5.0 G PPI dendrimers	10%	Bhadra et al. (2005)

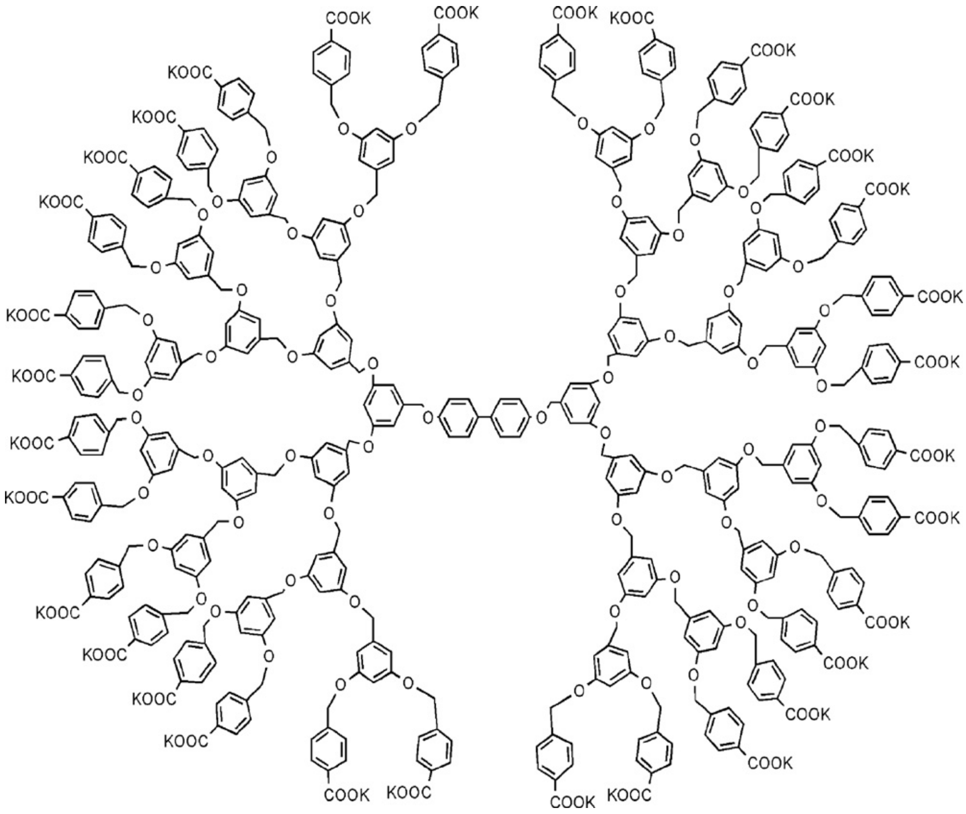

Fig. 6. Polyether dendrimer.

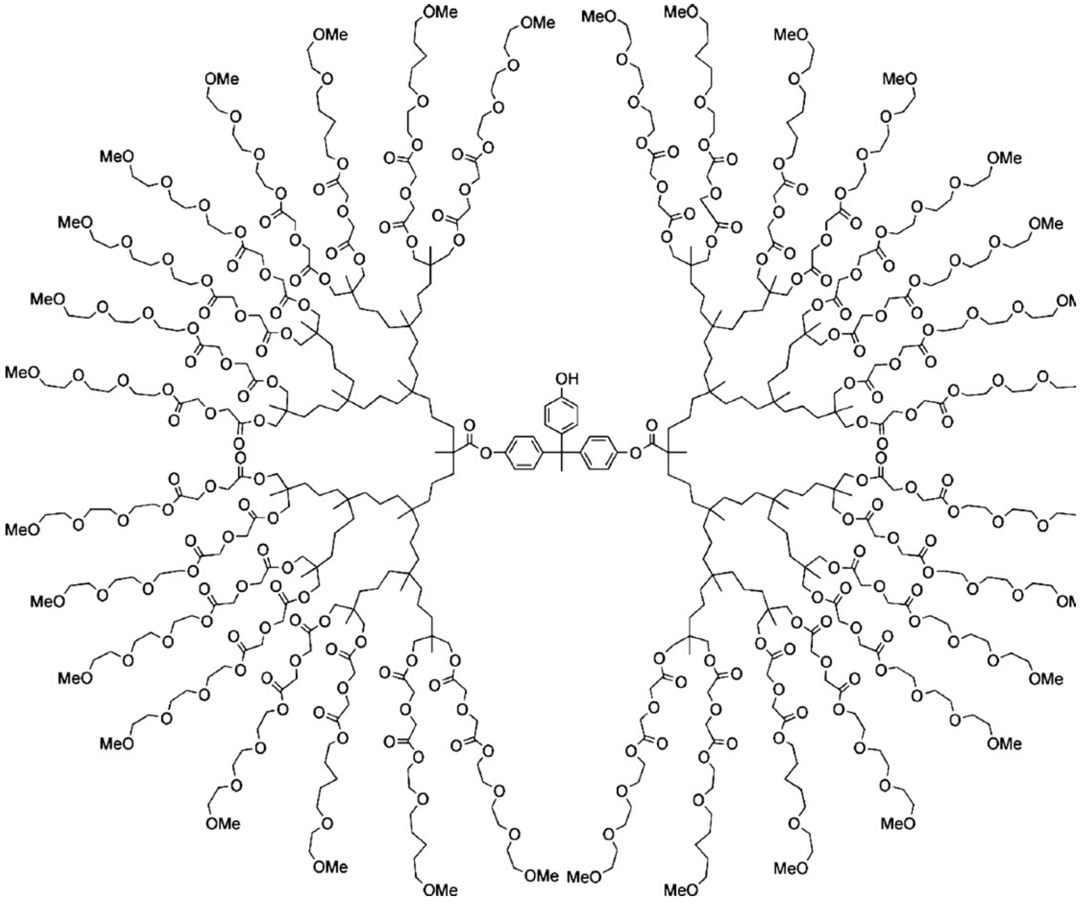

Fig. 7. Polyester dendrimer.

4.1.3. Polyether imine dendrimers

Krishna et al. (2005) performed the synthesis and biological evaluation of 3-amino-propan-1-ol based polyether imine dendrimers. They synthesized this dendrimer with nitrogen core using a new monomer 3-(bis-(3-hydroxypropyl)amino)propan-1-ol. The authors studied the *in vitro* cytotoxicity of these dendrimers with propyl ether-imine and carboxylic acid groups at their peripheries and carried out these studies by performing MTT assays on two different cell lines, human breast cancer T47D and African green monkey kidney CV-1. The cell survival rate was more than 98% up to 100 mg/ml of the dendrimer solutions in both the cell lines. The MTT assay of polyether imine dendrimers revealed no measurable cytotoxicity on both the cell lines.

4.1.4. Polyether–copolyester (PEPE) dendrimers

Carnahan and Grinstaff (2001) synthesized and characterized polyether–ester dendrimers from glycerol and lactic acid. Dhanikula and Hildgen (2007a) synthesized polyether–copolyester dendrimers from PEG and characterized their surface by AFM and X-ray photoelectron spectroscopy (XPS). They utilized these dendrimers for encapsulation and release of methotrexate and exploited methotrexate loaded dendrimers for the treatment of gliomas. Authors found enhanced efficacy and intratumoral transport capability with fine capacity to encapsulate methotrexate in dendrimeric architecture (Dhanikula and Hildgen, 2007b; Dhanikula et al., 2008). They evaluated the cytotoxicity of these PEPE dendrimers on RAW 264.7 cell lines using LDH leakage. These dendrimers were considered to be biocompatible and no death was reported up to concentration of 250 μg/ml (Dhanikula and Hildgen, 2007b). In addition to this, IC_{50} of methotrexate was reduced after loading into PEPE dendrimers. These dendrimers were also found to show enhanced permeability across blood brain barrier (BBB). Methotrexate loaded dendrimers were found to be effective against U87MG and U 343 MGa cells and methotrexate resistance cells. Dhanikula et al. (2008) concluded that these dendrimers may act as potential delivery systems for the treatment of gliomas.

4.1.5. Phosphate dendrimers

Domanski et al. (2004) investigated haemotoxicity and cytotoxicity of water-insoluble, 5.0 G thiophosphate (Fig. 8) dendrimers including their influence on human red blood cells structure, membrane integrity and on the growth of nucleated cells represented by Chinese hamster ovary (CHO-K1) cell line. Authors found increase in erythrocyte membrane stability and thermal durability but trigger in echinocytosis upon interaction of dendrimer with erythrocyte membrane proteins.

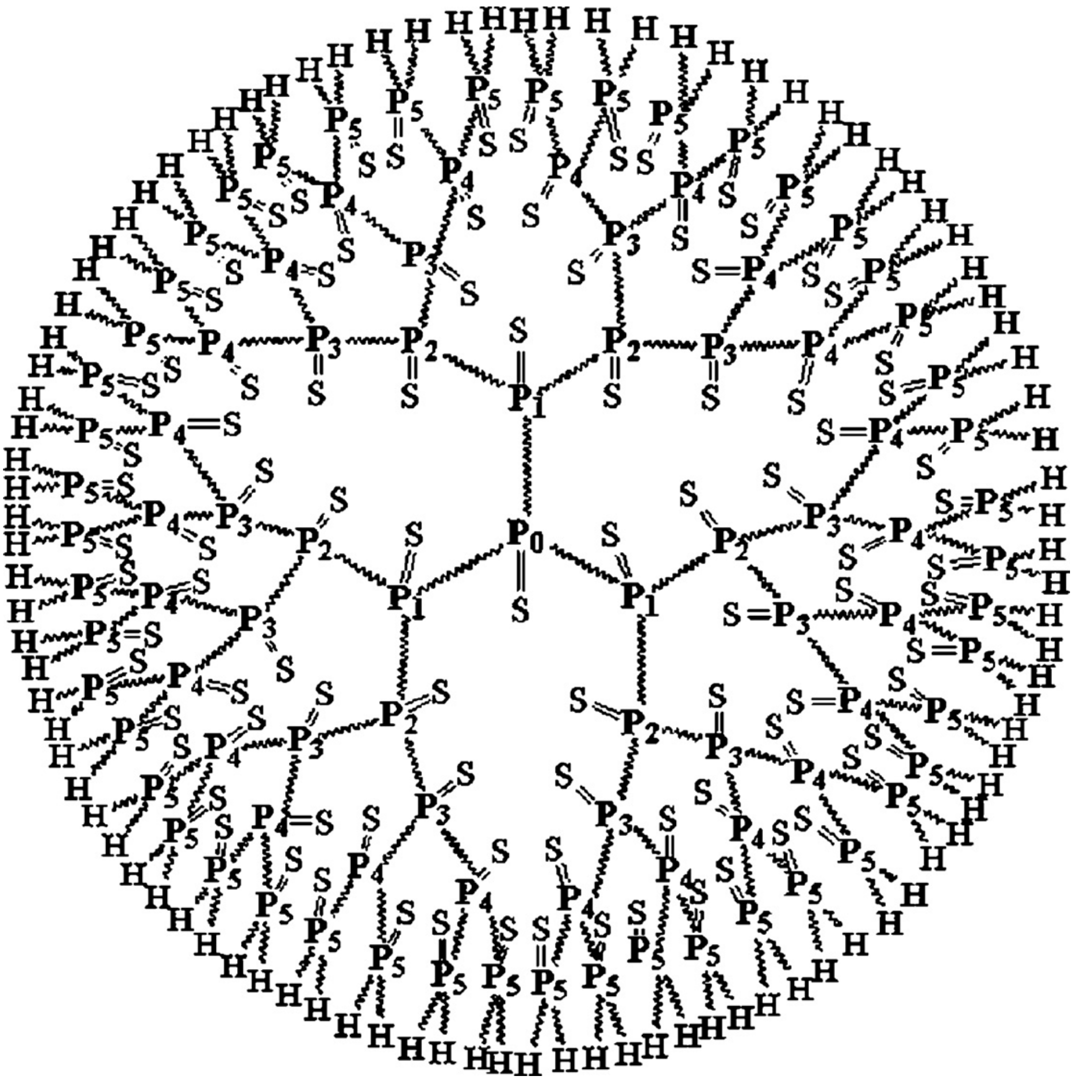

Fig. 8. Thiophosphate dendrimer.

4.1.6. Citric acid dendrimers

Namaji and Adeli (2005) synthesized triblock dendrimers of citric acid–polyethylene glycol–citric acid for drug delivery. The framework of dendrimers comprised of biocompatible materials. Authors solubilized some hydrophobic drug molecules like 5-amino salicylic acid (5-ASA), pyridine, mephenamic acid and diclofenac in aqueous solution. These drug–dendrimers complexes were found to be stable at room temperature for more than 10 months.

4.1.7. Melamine dendrimers

Melamine dendrimers were synthesized by both divergent and convergent growth method (Zhang and Simanek, 2000; Zhang et al., 2001). Multivalent dendrimers of melamine may be obtained by thiol-disulfide exchange (Umali and Simanek, 2003; Zhang et al., 2003a). These dendrimers were reported to reduce hepatotoxicity of anticancer drugs, methotrexate and 6-mercaptopurine with increased solubility by dendrimers (Neerman et al., 2008). These were investigated as drug delivery candidates and for *in vitro* and *in vivo* toxicity (Lim and Simanek, 2008; Chen et al., 2004; Neerman et al., 2004). Neerman et al. (2004) found that melamine dendrimer in doses up to 10 mg/kg showed no hepatic toxicity at subchronic doses although doses up to 40 mg/kg showed liver necrosis in mice.

4.1.8. Peptide dendrimers

This class of dendrimers comprises of different types of amino acids such as lysine, arginine etc and these dendrimers were found to exhibit promising vaccine and drug carrier properties (Agrawal et al., 2007; Wegmann et al., 2008; Cubillos et al., 2008; Tam and Spetzler, 2001). Shao and Tam (1995) developed peptide dendrimers using a new tactic with oxime, hydrazone, thiazolidine linkages using uprotected peptides as building blocks and selective ligation between an aldehyde and a weak base.

Agrawal et al. (2007) synthesized 4.0 G poly-L-lysine dendrimers using PEG 1000 as core and L-lysine amino acid as branching unit following repetitive protection with di-tertiary butyl pyrocarbonate (di-BOC) and deprotection step. They found a considerable reduction in hemolytic toxicity in comparison with PPI dendrimers of similar generation.

4.1.9. Triazine dendrimers

Simanek and coworkers made the major contribution in this regard. They assessed the propensity of triazine dendrimers as a drug delivery vehicle (Zhang et al., 2003b) and found these dendrimers a suitable solubilizing agent for hydrophobic molecules; carrier for delivery of drug molecule and apparently non-toxic in preliminary animal models. Triazine dendrimers was found to produce no toxic effect on kidneys and liver in single doses delivered intraperitoneally up to 10 mg/kg in preliminary toxicological studies in mice. Later, Chouai and Simanek (2008) reported a divergent and iterative synthesis method for 2.0 G triazine dendrimers (Fig. 9) for a kilogram scale using common laboratory equipment from trichlorotriazine. Lai et al. (2008) prepared star-shaped mesogens based on triazine and piperazine units. The biodistribution of triazine dendrimers is affected by number of PEG chains attached to the dendrimers while increase in molecular mass results in

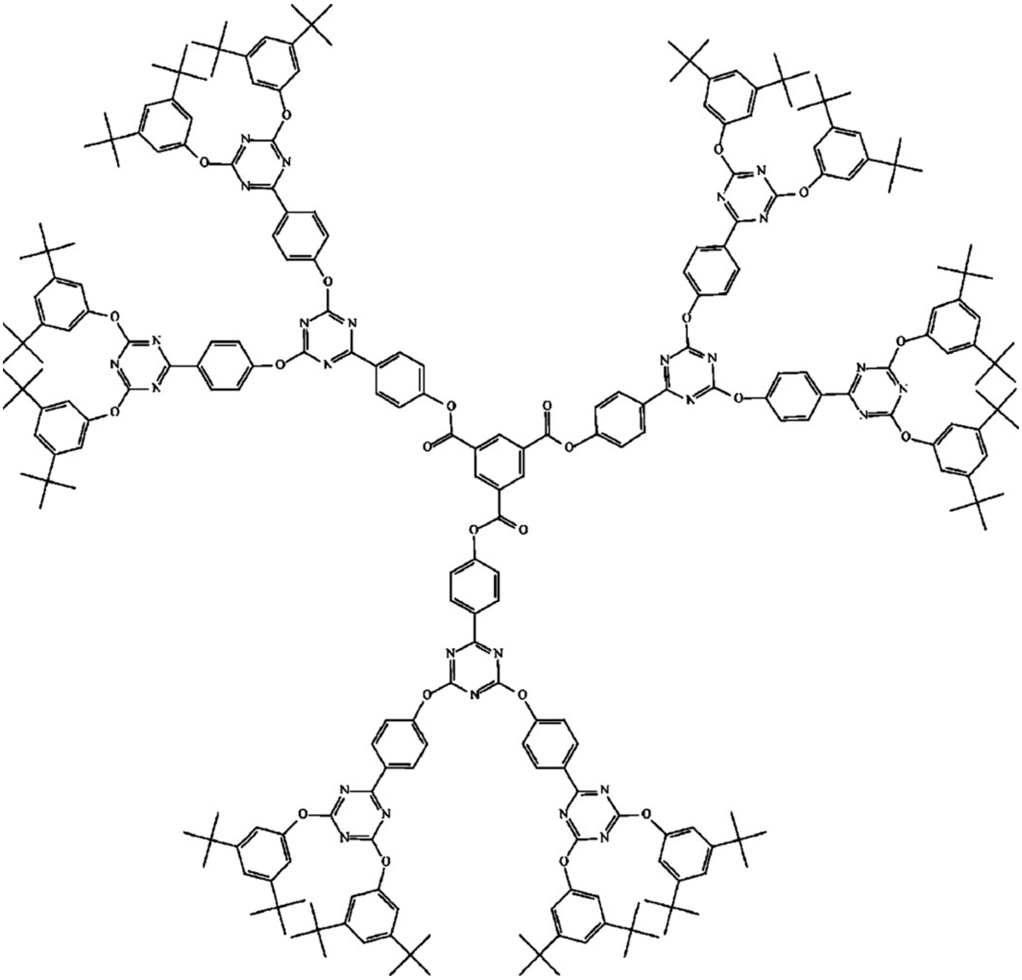

Fig. 9. Triazine dendrimer.

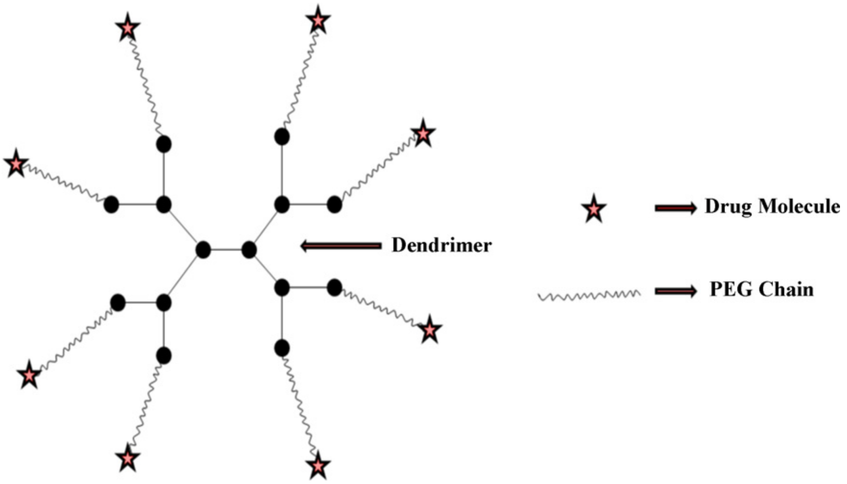

Fig. 10. Drug conjugated PEGylated dendrimer.

enhanced elimination half lives. Tumor uptake took place for all type of PEGylated dendrimers with multiple PEG groups of 0.6, 2, and 5 kDa (Lim et al., 2008). Recently, our group has developed triazine dendrimers using triazine trichloride as core. The dendrimers were developed using utilizing differential reactivity of the cyanuric chloride (triazine trichloride) upto third generation by divergent method using diethanolamine as branching unit. The studies related to drug delivery aspect of theswe dendrimers are in progress.

4.2. Surface engineered dendrimers

Surface engineering appears to be one of the best strategies for abatement of dendrimer toxicity (Tables 4–6). The presence of multiple surface sites also makes possible the attachment of moieties of various functionalities to the surface through covalent or non-covalent bonding. Surface modification of dendrimers leads to protection of surface amine groups and reduces the inherent cytotoxicity of dendrimers (Roberts et al., 1996; Jevprasesphant et al., 2003; Luo et al., 2002). Apart from reduction in inherent toxicity of dendrimers, functionalization also imparts some other properties beneficial for their use as drug delivery system including improvement in drug encapsulation efficiency, improved biodistribution and pharmacokinetic properties, increase in solubility, targeting to specific site, better transfection efficiency, sustained and controlled drug release, improvement in stability profile, and improved therapeutic potential as anti-viral, anti-bacterial activity (Gajbhiye et al., 2009; Bhadra et al., 2005, 2003; Konda et al., 2001; Agrawal et al., 2009).

4.2.1. PEGylation

One of the most important steps to reduce the cytotoxicity and haemolytic toxicity of dendrimers is to modify the surface amine/cationic groups with neutral or anionic moieties. Prevention of electrostatic interactions of dendrimers with cellular membranes apparently is a necessary step toward minimizing the toxicity of delivery vehicles to the endothelium (Stasko et al., 2007). PEGylation of dendrimers i.e. linking or conjugation of dendrimers with PEG (Fig. 10) is an important strategy in reduction of toxicity of dendrimers (Jevprasesphant et al., 2003; Stasko et al., 2007; Bhadra et al., 2003; Namaji and Adeli, 2005; Luo et al., 2002). In a breakthrough study Bhadra et al. (2003) explored PEGylation as a mean for reducing dendrimers toxicity and found that PEGylation of 4.0 G PAMAM dendrimers resulted in significant reduction in haemolytic- and haematological toxicity of uncoated PAMAM dendrimers with improvement in drug loading capacity and reduction in drug leakage. The authors also reviewed the PEGylation of various novel drug delivery systems including liposomes, drug, protein, enzymes and nanoparticles etc (Bhadra et al., 2002). Jevprasesphant et al. (2003) scrutinized the effect of surface modification of dendrimers on their cytotoxicity and found that surface engineering of PAMAM dendrimers with PEG and lauroyl chains resulted in appreciable reduction in the toxicity. In addition to reduction of cytotoxicity PEGylation also improves other limitations associated with dendrimers such as RES uptake, drug leakage, immunogenicity, stability and hence may result in significant improvement in drug therapy.

Various purposes of PEGylation of dendrimers include improved biodistribution and pharmacokinetics, increase in solubility of dendrimers, shielding of peripheral cationic groups to reduce toxicity (cytotoxicity and hemolytic toxicity), increase in drug loading to provide a better drug delivery system, sustained and controlled drug delivery, improved bioavailability/oral delivery application, better transfection efficiency, and tumor localization (Gajbhiye et al., 2009, 2007; Dufes et al., 2005; Bhadra et al., 2003).

4.2.2. Carbohydrate engineered dendrimers

Dendrimers that incorporate carbohydrate into their architecture are termed as ‘glycodendrimers’ (Fig. 11). These dendrimers may be classified as carbohydrate-centred, carbohydrate-based and carbohydrate-coated dendrimers (Agrawal et al., 2007). Mitchell et al. (1999) described two new methods for modification of PAMAM dendrimers with carbohydrates or peptides. These methods were based on oxime-forming condensation reaction between hydroxylamino nucleophiles and appropriate carbonyl compounds. Carbohydrate coating of dendrimers may provide several benefits over unconjugated dendrimers including reduction of haemolytic toxicity, cytotoxicity, immunogenicity, antigenicity by neutralization of peripheral cationic charges of dendrimers, site-specific delivery of drugs, ophthalmic delivery, gene therapy and also in diagnosis and treatment of cancer (Bhadra et al., 2005; Agrawal et al., 2007). For example galactose, a monosaccharide, is suitable for liver targeting due to the presence of asialo-glycoprotein receptors present on liver parenchymatous cells (Bhadra et al., 2005; Agrawal et al., 2007). Bhadra et al. (2005) conjugated galactose with PPI dendrimers and investigated their potential as carrier for liver targeting of primaquine phosphate. Authors evaluated haemolytic toxicity and effect on haematological parameters of dendrimer and found that these carbohydrate-coated dendrimers were biocompatible and resulted in significant

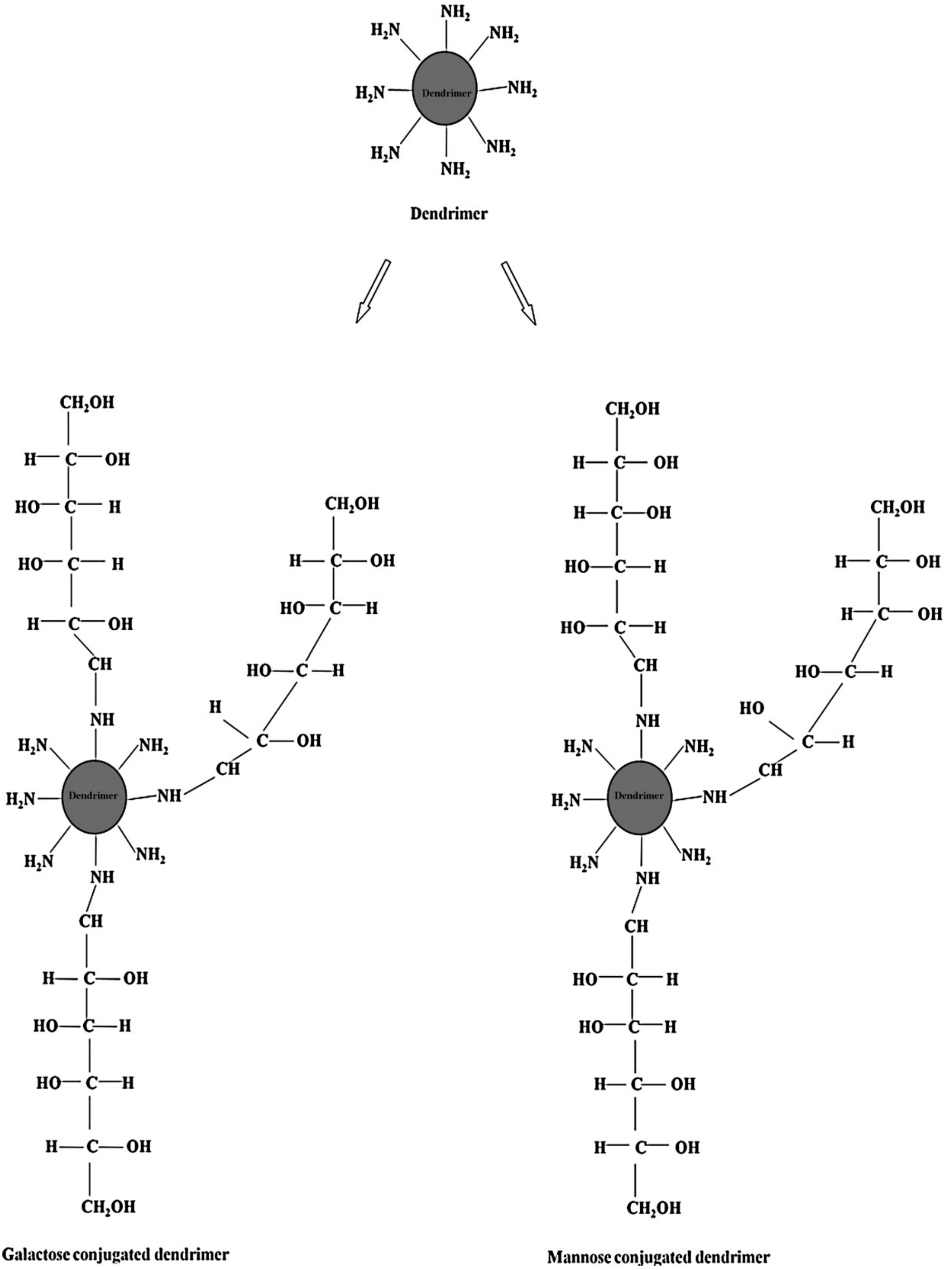

Fig. 11. Carbohydrate conjugated dendrimer.

reduction of haemolytic toxicity compared to uncoated dendrimers. Authors detected 35.7%, 49.2%. 10% and 7.1% hemolysis with 4.0 G, 5.0 G uncoated and 4.0 G, 5.0 G galactose coated PPI dendrimers, respectively, at concentration of 5 mg/ml after incubation for 1 h with RBC suspension at 37 °C. Similar results were obtained for the effect of plain and protected dendrimers on haematological parameters. A relatively higher stimulation of WBCs count and reduction in RBCs count by uncoated dendrimer compared with coated PPI dendrimers was obtained. Agashe et al. (2006) conjugated lactose and mannose to the 5.0 G PPI dendrimers and compared the toxicity profile of these dendrimers with that of the parent dendrimer. A substantial reduction in cytotoxicity and haemolytic toxicity was observed with sugar conjugated (lactose and mannose) dendrimers and statistically insignificant difference in haematological parameters was witnessed compared to the control. Thus, these carbohydrate-coated dendrimers could emerge as newer biocompatible carriers for drug delivery.

Polyether–copolyester dendrimers were conjugated with D-glucosamine as carrier for methotrexate in treatment of gliomas. They were evaluated for their efficacy against U87 MG and U 343 MGa cells, permeability across *in vitro* BBB model and distribution into avascular human glioma tumor spheroids. These glucosylated dendrimers were found effective against gliomas, more permeable across BBB and able to kill even methotrexate resistant cells, including reduction of IC_{50} of methotrexate. In distribution studies with glioma tumor glucosylated dendrimers were found to be distributed throughout the avascular tumor spheroids within 6 h, while nonglucosylated dendrimers could do so in 12 h (Dhanikula et al., 2008). Agrawal et al. (2007) synthesized galactose coated 4.0 G poly-L-lysine dendrimers and reported 5.2 times reduction in the hemolytic toxicity compared to 4.0 G PPI dendrimers coupled with a negligible effect on hematological parameters.

4.2.3. *Acetylation*

As discussed above, neutralization of the amine surface charge of dendrimer is essential to preclude toxicity and nonspecific uptake of the drug conjugates and the modification of surface amino groups of dendrimers with acetyl groups has resulted in the reduction of toxicity of dendrimers (Stasko et al., 2007). Plain PPI dendrimers demonstrated prominent cytotoxicity in HUVEC, but when Stasko et al. (2007) prepared fluorescently labeled derivatives of 5.0 G PPI dendrimers with acetylation and evaluated their cytotoxicity and membrane disruption in HUVEC cells, they found a significant reduction in the acute cytotoxicity of cationic primary amine containing dendrimers.

Acetylation of dendrimers is also a route to specifically functionalize the primary surface amines (Zhuo et al., 1999). Zhuo et al. synthesized a series of dendritic polymers with a core of 1, 4, 7, 10-tetraazacyclododecane and Michael addition of methyl acrylate followed by amidation with ethylenediamine. They then acylated the peripheral amine groups by acetic anhydride, which leads to increased solubility of dendrimers in water. The acetylated dendrimers were then reacted with 1-bromoacetyl-5-fluorouracil to form dendrimer-5-FU conjugates to form a carrier for the controlled release of antitumor drugs (Zhuo et al., 1999). Recently Waite et al. (2009) studied the acetylated 5.0 G PAMAM dendrimers for cellular delivery of siRNA. PAMAM dendrimers were acetylated with acetic anhydride and complexed with siRNA, the cytotoxicity of complex was evaluated using U87 malignant glioma cells and increase in acetylation resulted in reduced polymer cytotoxicity to U87 cells with enhanced dissociation of dendrimer/siRNA complexes and thus promoted the release of siRNA from complexes.

Majoros et al. (2002) have defined the exact percentage of acetylated end groups of the dendrimer with several techniques including potentiometric titration, GPC, ^{1}H NMR, and ^{13}C NMR and investigated the precise stoichiometry required for the acetylation of surface amines of 5.0 G PAMAM dendrimer, the nature of the acetylation reaction used and an analysis of well-defined dendrimer conjugates for use in the biomedical field.

Kolhatkar et al. (2007) acetylated the amine groups of PAMAM dendrimers and investigated the effect of this modification on cytotoxicity, permeability and cellular uptake in Caco-2 cell monolayers. Authors found a 10-fold decrease in cytotoxicity with increase in number of surface acetyl groups while maintaining permeability across plasma membrane.

Kukowska-Latallo et al. (2005) conjugated the acetylated PAMAM dendrimers with folic acid as a targeting agent and then coupled these conjugates to methotrexate or tritium and either fluorescein or 6-carboxytetramethylrhodamine. They injected these conjugates in mice bearing human KB tumors that overexpress the folic acid receptor and found a 10-fold higher efficacy with respect to free methotrexate. No gross toxicity was observed up to 99 days and also no morphologic abnormalities were observed on the histopathological examination of the liver, spleen, kidney, lung and heart.

4.2.4. *Half generation- or anionic dendrimers*

Dendrimers like PAMAM and PPI have positively charged surface groups after full generation synthesis, but their half generations have carboxylic acid surface groups or cyanide surface groups, respectively (Fig. 12). We have already discussed that the toxicity of dendrimers and other cationic molecules is due to their surface cationic groups and hence half generation dendrimers, which has negatively charged carboxylic or cyanide surface groups, shows negligible toxicity (haemolytic toxicity or cytotoxicity) (Malik et al., 2000; Roberts et al., 1996; Bhadra et al., 2003). Malik et al. (2000) reported that carboxylic acid-terminated PAMAM dendrimers were not haemolytic up to concentration of 2 mg/ml, whereas amine-terminated full generation PAMAM were found to be lytic above a concentration of 1 mg/ml. Bhadra et al. (2003) also reported similar results, and found that half generations of carboxylic acid-terminated PAMAM dendrimers showed negligible hemolytic toxicity while full generation PAMAM dendrimers showed a haemolysis of ~15.3–17.3%. Jevprasesphant et al. (2003) also studied the cytotoxicity of whole generation (cationic) and half generation (anionic) PAMAM dendrimers in Caco-2 cells and found a higher toxicity for whole generation dendrimers compared to half generation (Jevprasesphant et al., 2003). Thus half generation dendrimers are lesser or non-toxic as compared to full generations. These reports hold valid for PAMAM dendrimers and may be applicable to other dendrimer types as well.

4.2.5. *Amino acid or peptide conjugated dendrimers*

Amino acid such as phenyl alanine and glycine conjugated dendrimers show a significant reduction in toxicities associated with dendrimers (Agashe et al., 2006). Conjugation of these amino acids to 5.0 G PPI dendrimers revealed a substantial reduction in haemolytic toxicity; also these amino acid functionalized dendrimers did not demonstrate any concentration- and time-dependent cytotoxicity. Many techniques have been employed for decoration of dendrimers with amino acids and peptides at their surface. Kono et al. (2005) tailored PAMAM dendrimers with phenylalanine or leucine residues at their periphery to attain efficient gene transfection. Authors prepared phenylalanine- or leucine-grafted PAMAM dendrimers by reaction of amine-terminated 4.0 G PAMAM dendrimers with butyl pyrocarbonate protected phenyl alanine (*Boc-Phe*) or butyl pyrocarbonate protected leucine (*Boc-Leu*) using N-hydroxy succinimide (NHS) and N,N′-dicyclohexyl carbodiimide (DCC). They found higher transfection efficiency in addition to lower cytotoxicity with phenylalanine-modified dendrimers in contrast with some commonly applied transfection agents.

Arginine conjugated dendrimers demonstrated higher transfection efficiency and suitability for gene delivery (Choi et al., 2004; Okuda et al., 2004). Dendrimers have also been modified with some peptides (Fig. 13). Yang and Kao (2007) conjugated starburst anionic 3.5 G and cationic 4.0 G PAMAM dendrimers with arginine-glycine-aspartate (RGD) peptides. They monitored cellular internalization of dendrimers by utilizing FITC-conjugated PAMAM dendrimers in adherent fibroblasts and reported that anionic 3.5 G-based dendritic RGD clusters showed no negative effect on fibroblast viability as well as for cationic 4.0 G-based dendritic RGD clusters at lower concentrations. According to authors these nanoscale dendritic RGD clusters hold great potential for

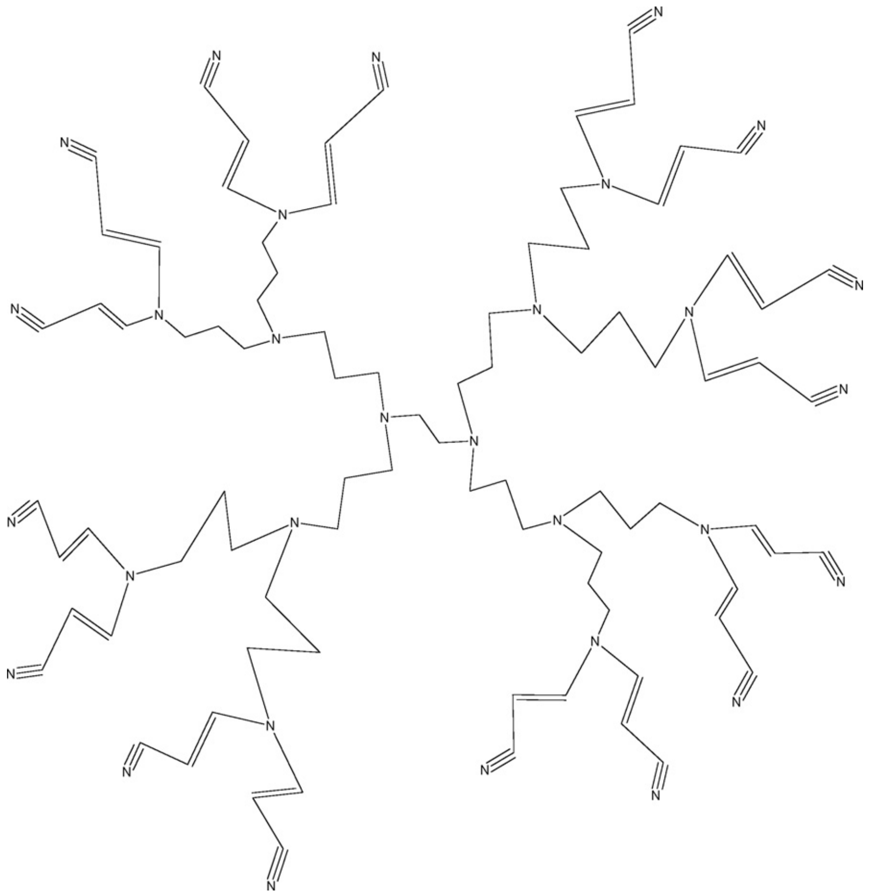

Fig. 12. 2.5 G PPI dendrimers with negatively charged cyanide surface groups.

tissue engineering as well as in drug delivery (Yang and Kao, 2007).

4.2.6. Drug and DNA/gene conjugated dendrimers

Drug conjugation to dendrimer surface may also result in reduction of toxicity of dendrimers (Fig. 14). Kolhatkar et al. (2008) complexed SN-38 (7-ethyl-10-hydroxy-camptothecin) with 4.0 G PAMAM dendrimers to improve oral bioavailability of SN-38, a biologically active metabolite of irinotecan hydrochloride. Irinotecan hydrochloride, an anticancer agent, has oral bioavailability of about 8% only and displays gastrointestinal toxicity. SN-38 has potent antitumor activity i.e. approximately 1000-fold more active than irinotecan hydrochloride. Authors performed this study to investigate the potential of PAMAM in improving the delivery of SN-38. They synthesized the complex of SN-38 with PAMAM dendrimers and evaluated its stability, permeability and cellular uptake by Caco-2 cells and found that the complex was stable at pH 7.4, and drug was released at pH 5.0. A 10-fold increase in permeability

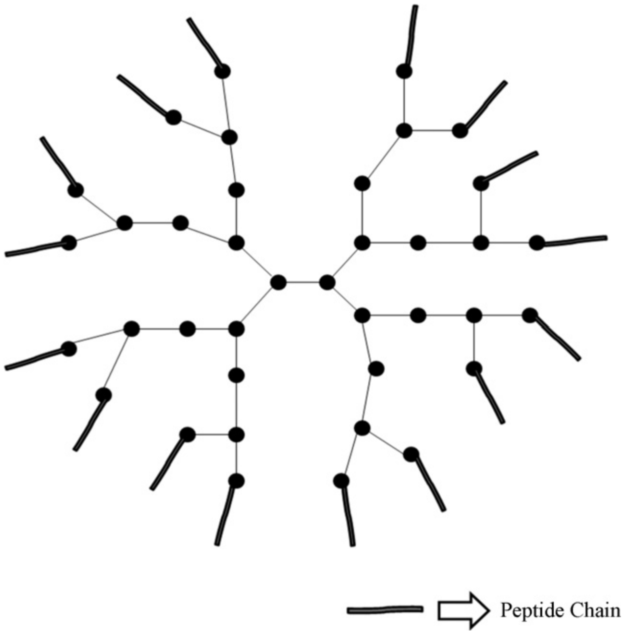

Fig. 13. Peptide conjugated dendrimers.

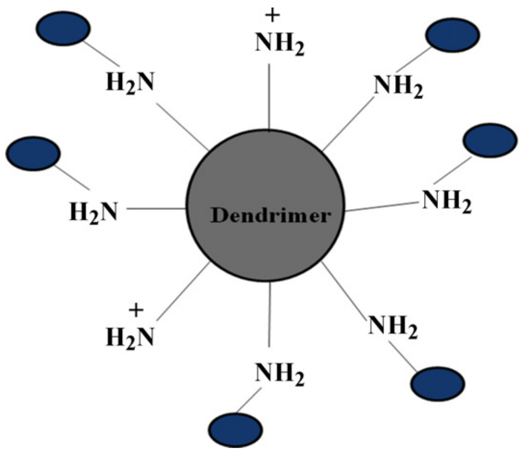

Fig. 14. Drug molecules (●) conjugated with dendrimer.

Fig. 15. Formation of complex between DNA and dendrimer via interaction of positive and negative charge.

and more than 100-fold increase in cellular uptake was observed with respect to free SN-38. In cytotoxicity studies with complex no appreciable toxicity was observed after incubation for 2 h with Caco-2 cells at 0.1 μM concentration. Therefore dendrimer drug complex may be used to reduce the cytotoxicity of dendrimers with improvement in stability, drug delivery, permeability and cellular uptake (Lee et al., 2006).

Our group has shown that complex of 4.0 G PAMAM dendrimer with water-insoluble and acidic anti-inflammatory drug, flurbiprofen, results in lesser haemolytic toxicity at an equivalent concentration than the plain dendrimers. This is because drug molecules could protect the peripheral free amino groups of dendrimers (Asthana et al., 2005). Similarly complexation of dendrimers with DNA or gene also results in reduction of cytotoxicity of dendrimers because of protection of cationic charge of dendrimers (Li et al., 2007). Complexation of dendrimer with DNA or oligonucleotides (Fig. 15) leads to significant reduction in toxicity of dendrimers and this is due to the protection of cationic charge of dendrimers by negatively charged DNA. Many researchers have found dendrimer–DNA or dendrimer–oligonucleotide complexes less cytotoxic than the parent dendrimers (Brazeau et al., 1998; Yoo and Juliano, 2000).

4.2.7. *Antibody functionalized dendrimers*

Antibody conjugated delivery systems are a novel strategy for the delivery of cytotoxic and other therapeutic agents to specific cell types. Antibodies may be a very important tool in cancer targeting as they may serve as a homing device to deliver therapeutic agents to tumor site, may lead to specific targeting of tumor cells that overexpresses certain antigens (Chang et al., 1999), may target and attack the blood vessels feeding a tumor or the connective tissues (stroma) supporting it i.e. antiangiogenic effect (Wu et al., 2006), or they may block or neutralize the action of growth factors required for the growth of tumor cells. Dendrimers have also been conjugated with antibodies to form immunoconjugates to serve various therapeutic needs. Two different antibodies 60bca and J591 that bound to CD14 and prostate specific membrane antigen (PSMA), respectively conjugated to 5.0 G PAMAM dendrimers and labeled with fluorescein isothiocyanate (G5-FI-60B, G5-FI-PA) for targeting CD14-expressing HL-60 human myeloblastic leukemia cells and PSMA expressing LNCaP prostate cancer cells. The antibodies 60bca and J591 binds to CD14 and prostate specific membrane antigen (PSMA), respectively. Thomas et al. (2004) conjugated these two antibodies (60bca and J591) to 5.0 G PAMAM dendrimers for targeting CD14-expressing HL-60 human myeloblastic leukemia cells and PSMA expressing LNCaP prostate cancer cells and labeled these antibody conjugated dendrimers with fluorescein isothiocyanate (G5-FI-60B, G5-FI-PA). Flow cytometry, confocal microscopy and two-photon-based optical fiber fluorescence (TPOFF) detection system showed the uptake of conjugates to specific antigen expressing cells while control G5-FI lacked specific affinity for any of the cell lines (Thomas et al., 2004). Similarly Shukla et al. (2006) synthesized anti-HER2-G5-PAMAM dendrimers tagged with AlexaFluor (AF) for targeting HER2-expressing cells. This conjugate was synthesized for the targeting of human growth factor receptor-2 overexpressing breast and ovarian cancer cells. The conjugate not only showed the binding and internalization into HER2-expressing MCA-207 cell lines but also it targeted the HER2-expressing tumors in *in vivo* studies with animals.

Patri et al. (2004) synthesized immunodendrimers by tailoring PAMAM dendrimers with J591 anti-PSMA monoclonal antibody and observed that these conjugates specifically bind to cells expressing PSMA. A monoclonal antibody cetuximab, which binds to epidermal growth factor receptor (EGFR), was covalently linked to 5.0 G PAMAM dendrimers via its *Fc* region containing drug methotrexate for targeting EGFR-positive brain tumors. The conjugate was found to be cytotoxic with specific affinity to EGFR-expressing rat glioma cell lines F98 (EGFR) (Wu et al., 2006). Wangler et al. (2008) examined the impact of conjugation on immunoreactivity of derivatized antibody with respect to size and number of conjugates and for this they conjugated an anti-EGFR antibody hMAb425 with PAMAM dendrimers of different size containing up to 128 chelating agents per conjugation site and concluded that the number of derivatization sites had crucial effect on immunoreactivity whereas dendrimer size did not have significant influence on activity.

4.2.8. *Tuftsin conjugated dendrimers*

Tuftsin is a natural macrophage activator tetrapeptide (*Thr-Lys-Pro-Arg*) produced by enzymatic cleavage of the *Fc*-domain of heavy chain of immunoglobulin G as the free peptide fragment (Najjar, 1987), first identified by Najjar and Nishioka (1970). It binds to specific receptors on macrophages, monocytes and polymorphonuclear leukocytes and possesses a broad spectrum of activities related primarily to the immune system. It stimulates the migration, phagocytic, bactericidal and tumoricidal activity of macrophages and leukocytes (Fridkin and Najjar, 1989; Agrawal and Gupta, 2000). Since tuftsin specifically binds monocytes, macrophages and polymorphonuclear leukocytes and potentiates their natural killer activity against tumors and pathogens; it may be used to increase drug specificity and lower systemic drug toxicity by targeting drug to these cells (Gupta and Haq, 2005; Bai et al., 2008). It has been conjugated with liposomes to increase the uptake of amphotericin B by macrophages to improve chemotherapeutic efficacy of amphotericin B for treatment of leishmanial infections (Gupta and Haq, 2005; Agrawal et al., 2002). Tuftsin was also conjugated with an anti-viral drug Azidothymidine (AZT) to target HIV-infected macrophages (Fridkin et al., 2005). It was attached with an anticancer drug methotrexate (MTX) through an enzyme-labile pentapeptide spacer (GFLGC) to form chemotactic targeting moiety for targeting cancer chemotherapy (Bai et al., 2008). Mezo et al. (2006) synthesized oligotuftsin-based branched oligopeptide for chemotactic drug targeting. They prepared tetratuftsin derivatives containing chemotactic formyl tripeptide conjugated with methotrexate and studied their chemotactic activity and cytotoxicity.

In 2008 Dutta et al. prepared efavirenz, an anti-viral drug, loaded tuftsin conjugated PPI dendrimers (TuPPI) for targeting HIV-infected macrophages *in vitro* and observed a much higher cellular uptake of efavirenz from TuPPI compared to free drug by HIV-infected fresh human mononuclear cells (Mo/Mac). They also evaluated the cytotoxicity of TuPPI in these cell lines and found a significant reduction in cytotoxicity of PPI dendrimers ($p < 0.001$). This reduction in cytotoxicity was thought to be due to the shielding effect of the bulky peptide masking the cytotoxic free primary amino groups at the periphery of PPI dendrimers and thus preventing the amino groups from interacting with the cell membrane and damaging them (Dutta et al., 2008).

4.2.9. Folate-conjugated dendrimers

The folic acid receptor is overexpressed by many types of tumor cells such as lung, renal, ovarian, endometrial, colorectal, breast, testis, myelocytic blood cells, neuroendocrine carcinomas and brain metastases (Garin-Chesa et al., 1993; Wang and Low, 1998). Folate-anchored carriers enter the cell by receptor mediated endocytosis (RME), bypassing cellular barriers, and allow the attached moiety to enter cancer cells of tumor xenografts (Caliceti et al., 2003). Folate mediated targeting has not been used not only in drug delivery but also for the delivery of gene, imaging agent and diagnostic agents (Wang and Low, 1998). This phenomena has also been utilized in dendrimer mediated cancer therapy and folate-conjugated dendrimers were developed to target tumor cells (Singh et al., 2008; Quintana et al., 2002). Kono et al. (1999) synthesized folate-anchored polyether dendritic systems as a carrier with potential tumor cell specificity. They prepared this carrier system by introducing hydrazide groups to ester-terminated polyether dendrimers by reaction with hydrazine followed by conjugation of folate residues to the hydrazide chain ends of the dendrimers by direct condensation with folic acid. These conjugates were soluble in aqueous medium above pH 7.4 (Kono et al., 1999). PAMAM dendrimers were conjugated with FITC and then with folate through EDC linkage. 5.0 G PAMAM dendrimer was dissolved in DMSO and sodium phosphate buffer under nitrogen for conjugation to FITC. The mixture was stirred in the dark for ~24 h, then washed and ultrafiltered in Centricon tubes. Furthermore, Quintana et al. (2002) conjugated folate to the dendrimer through an EDC linkage. An anticancer drug (methotrexate) was incorporated into dendrimers aimed at effective delivery to the cancer tissues with reduced toxicity. To gain access to the tumor surface, via the circulatory system, the drug needs to be hydrophilic; and to penetrate the cell membrane, the lipid solubility must be high. The simultaneous occurrence of both properties in the drug is difficult and, as discussed previously, folate-conjugated dendrimers enter the cell by RME, bypasses the cellular barriers, allowing hydrophilic drugs to enter cancer cells of tumor xenografts (Quintana et al., 2002). Later, Choi et al. (2005) also utilized this method to conjugate folic acid or FITC to partially acetylated PAMAM dendrimers and finally they attached this system covalently to oligonucleotides. In *in vitro* studies this DNA-linked dendrimer clusters indicated specific binding to KB cells expressing the folate receptor and also showed the internalization of the dendrimer cluster. Singh et al. (2008) conjugated PAMAM dendrimers with folic acid and folic acid–PEG conjugates and evaluated their efficacy and safety in tumor-bearing mice and found these folate-PEG-dendrimer to be significantly safe and effective in tumor targeting including reduction in hemolytic toxicity, sustained release and higher accumulation in tumor area.

4.2.10. Miscellaneous

Baker and co-workers synthesized PAMAM dendrimer based multifunctional nanodevice for delivery of anticancer agent. On to these multifunctional dendrimers authors combined the cancer imaging and targeted delivery by attaching different ligands; acetyl group to neutralize primary amine groups, FITC for cancer imaging, folic acid as targeting moiety and methotrexate, an anticancer agent (Majoros et al., 2005).

Miao et al. (2007) incorporated biodegradable poly(ε-caprolactone)s onto the PAMAM dendrimers. The authors synthesized a series of star poly(ε-caprolactone)s (PCL) with dendritic cores via ring-opening polymerization of ε-caprolactone initiated by PAMAM dendrimers. The *in vitro* degradation of these dendrimers is influenced by the content of PAMAM dendrimers and caprolactone. Thus a highly biodegradable carrier may be obtained by incorparing caprolactone with dendrimers.

Li et al. (2007) synthesized amphiphilic dendritic poly(L-lysine)-b-poly(L-lactide)-b-dendritic poly(L-lysine) dendrimers bearing two two-generation of poly(L-lysine) dendrons and a central hydrophobic biodegradable poly(L-lactide). These dendrimers was designed, synthesized and applied as a biodegradable gene carrier. Their cytotoxicity was appraised by conducting MTT assay in human hepatocellular carcinoma cell line (SMMC-7721) and a very low cytotoxicity was observed (Li et al., 2007).

5. Conclusion and future prospects

The past decade witnessed a large explosion in the field of drug delivery. A number of delivery devices were explored to control drug disposition in the biological environment in order to obtain maximum therapeutic benefit with minimum toxicity. Along with these delivery devices dendrimer has emerged as promising drug delivery device in modern medicine system. Their well-defined architecture made them useful in biological systems. Dendrimers with their large molecular size and multivalent surfaces provide an excellent platform for the attachment of drug, gene, targeting moieties and homing devices, imaging agents etc., as well as they can be hydrophobically modified to mimic inverse micelles and have presented unrestrained ‘guest host’ properties.

Although dendrimers has made tremendous progress in the delivery of therapeutic agents yet their posbile potential is constrained because of inherent toxicity problems associated with them. Dendrimers and other cationic macromolecular delivery system interact nonspecifically with negatively charged biological membrane. This non-selective interaction of cationic delivery system causes membrane disruption through formation of nanoholes, membrane thinning and membrane erosion followed by leakage of cytosolic enzymes and cell death. Consequently it results in different toxicities of dendrimers including cytotoxicity, haemolytic toxicity and haematological toxicity. Currently, there is great need to devise strategies to overcome difficulties encountered in drug delivery using a parent dendrimer as the carrier.

A plethora of research is going on to resolve the issues relating to safety, toxicity and efficacy of dendrimers in order to formulate some strategies for reduction of toxicity associated with dendrimers. Design and development of new dendrimers including biodegradable and/or biocompatible and surface engineered dendrimers may alleviate this problem successfully and may lead to a delivery system with a potential therapeutic efficacy *vis a vis* minimum biological toxicity. The major emphasis is on the development of biomimetic dendrimers, which will enhance the biocompatibility of dendrimers. A detailed study of these nanostructures would help in the evolution of newer concepts that could be intimately involved in sustaining the life processes. Various dendrimers having framework of biocompatible materials have been developed in the last decade such as citric acid dendrimers composed of citric acid, and polyethylene glycol, phosphate dendrimers, lysine dendrimers etc. Some other biodegradable dendrimers including polyester dendrimers, polyether dendrimers, triazine dendrimers have also been developed to reduce the toxicity of dendrimers.

Next to the development of biocompatible dendrimers, another rewarding strategy for the development of less toxic dendrimer is surface engineering. In case of dendrimers surface engineering is a method of modifying the surface of dendrimers with various functional groups to mask the cationic charge of dendrimers. It is the method by which cytotoxic dendrimers could be converted into biocompatible dendrimers. This has been supported by various surface engineering strategies such as PEGylation, acetylation, folate conjugation, carbohydrate, peptide, drug, gene and tuftsin conjugated, anionic and antibody functionalized dendrimers. Besides reduction of toxicity surface engineering may also accomplish some other benefits in the field of drug delivery including drug encapsulation, solubilization, protection of biomolecules, gene transfection, modified release, targeting with improved stability profile, biodistribution and therapeutic potential. Finally, surface engineering is an important tool in further exploration of pharmaceutical and biomedical potential of dendrimers.

The future of dendrimer as drug delivery module relies on fine tuning of three component of dendrimer architecture i.e. core, branches and surface groups which made these carrier inimitable amidst existing nanoscale carrier system. It is expected that new methods and strategies for synthesizing biocompatible dendrimers will be developed. Dendritic boxes, which have been studied for their carrier properties, may also emerge useful in reducing toxicity of dendrimers. Unfortunately there are only few reports on the *in vivo* toxicity as well as *in vivo* performance of these wonderful carriers and hence it is expected that more focused experiments will be performed in this direction.

The key to the development of safe dendrimers lies in fine tuning the nature and quantum of surface charge through the ingenious strategies known to the pharmaceutical scientist thoroughly exposed to drug delivery vistas. Attainment of GRAS status to nanoscopic dendrimers may revolutionise the modern drug delivery scenario.

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