CHAPTER

8

Dendrimers as novel drug-delivery system and its applications

Vruti Patel[1,*], *Chitra Rajani*[1,*], *Debleena Paul*[1], *Pooja Borisa*[1,*], *Kuldeep Rajpoot*[2], *Susanne R. Youngren-Ortiz*[3] *and Rakesh K. Tekade*[1,4]

[1]National Institute of Pharmaceutical Education and Research (NIPER) Ahmedabad, Gandhinagar, India [2]Pharmaceutical Research Project Laboratory, Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur, India [3]Akorn Pharmaceuticals, Inc., Research and Development, Vernon Hills, IL, United States [4]Department of Materials Engineering, Indian Institute of Technology (IIT) Jammu, Jammu, India

OUTLINE

8.1 Introduction 334
8.1.1 Specific properties of dendrimers 338
8.1.2 Disadvantages 341
8.1.3 Synthesis of dendrimers 341
8.2 Classes of dendrimer 343
8.2.1 Polyamidoamine dendrimer 344
8.2.2 Glycodendrimers 348
8.2.3 Liquid crystalline dendrimers 349
8.2.4 Peptide dendrimers 349
8.2.5 Chiral dendrimers 352
8.2.6 Hybrid dendrimers 352
8.2.7 Polyamidoamine organosilicon dendrimer 353
8.2.8 Core–shell tectodendrimers 355
8.2.9 Poly(propylene imine) dendrimers 356
8.2.10 Triazine dendrimers 357
8.2.11 Polylysine dendrimers 358
8.3 Types of dendrimers 358
8.3.1 Drug-loaded dendrimer as nanovehicles 358
8.3.2 Simple dendrimers in gene transfection 360

* Authors having equal contribution and can be interchangeably regarded as the first author.

DOI: https://doi.org/10.1016/B978-0-12-814487-9.00008-9

8.3.3 PEGylated dendrimers in cancer therapy 362
8.3.4 Liposomal "locked in" dendrimer 363
8.3.5 Targeted dendritic scaffolds 365
8.4 Applications of dendrimers 370
8.4.1 Drug delivery 370
8.4.2 Oligonucleotide labels 372
8.4.3 Photodynamic therapy 372
8.4.4 Gene delivery 373
8.4.5 Boron neutron capture therapy 373
8.4.6 Small interfering ribonucleic acid delivery 374
8.4.7 Oligonucleotide delivery 376
8.4.8 Targeted delivery 376
8.4.9 Vaccine delivery 377
8.4.10 Tissue engineering 378
8.4.11 Imaging 378
8.4.12 Dendrimer as drug 380
8.4.13 Nonmedical applications of dendrimers 381
8.4.14 Dendrimer-based products 382
8.5 Conclusion 383
Disclosures 384
Abbreviations 384
Acknowledgments 385
References 385
Further reading 392

8.1 Introduction

Several pharmacologically active drugs report some difficulties such as poor water solubility, low half-life, lack of specificity, and biocompatibility issues. To overcome these disputes as well as to improve the release characteristics of these drugs, several approaches are employed, that is, products prepared in crystalline solid forms, amorphous forms, lipid-based formulations, polymer–drug conjugates. However, these technologies are associated with some stability and toxicity issues (Madaan et al., 2014). Therefore nanoparticles-assisted drug delivery has been extensively explored in recent times in delivering and targeting therapeutics, and diagnostic agents together in a single system, which uses biodegradable and biocompatible polymers. Polymers exhibit few unique properties such as enhanced drug solubility, drug targeting ability, and biocompatibility. Hence, the advances in polymer science and its implementation in advanced drug delivery have witnessed their widespread applications (Kim et al., 2009). Broadly polymers can be classified into four types (1) linear, (2) cross-linked with side-chains or side-functional groups, (3) branched, and (4) perfect polymers. Traditional linear polymers, namely, polyethylene glycol (PEG), polyglutamic acid, polysaccharide, poly(allylamine hydrochloride), and *N*-(2-hydroxypropyl) methyl acrylamide have been reviewed as drug-delivery vehicles and accepted for clinical use, but these linear polymers have poorly defined chemical structures (Table 8.1) (Madaan et al., 2014).

Dendrimers are hyperbranched, well-defined structures in the range of nanosize (1–100 nm), globular in shape, low polydispersity macromolecules (typically $5000 \times 500{,}000$ g/mol) (Madaan et al., 2014). It is only an architectural motif (Abbasi et al., 2014). The term dendrimer is adopted from the Greek word dendron meaning "tree" due to its morphological structure simulating that of the tree branches and meros meaning "part." They are also known as "cascade molecules," "arborols" (a Latin word "arbor" meaning a "tree") (Abbasi et al., 2014), "dendritic molecules," or "nanometric architectures." The first

TABLE 8.1 Properties of dendrimers versus linear polymer.

Property	Dendrimeric architecture	Linear polymers
Architecture	Compact and globular architecture	Noncompact
Synthetic route	Stepwise growth	Single-step polycondensate strategy
Structural control	Very high uniformity	Low
Architecture	Regular in nature	Irregular
Shape	Spherical assembly	Random coil
Crystallinity	Noncrystalline, amorphous material	Semicrystalline/crystalline material
Water solubility	High	Low
Nonpolar solubility	High	Low
Solution viscosity	Nonlinear relationship with a molecular weight	Linear relation with a molecular weight
Reactivity	High, depending on surface functionality	Usually low
Compressibility	Low	High
Polydispersity	Monodisperse in nature	Polydisperse assemblies

dendrimer-like compound, poly(propylene imine) (PPI) with the low generation, was reported by Fritz Vogtle et al. in 1978 by divergent technology and by Donald Tomalia et al. in the early 1980s (Abbasi et al., 2014; Patri et al., 2005).

Dendrimers comprise layers of dendrons (branching units) emanating from a central initiating core with each layer forming a generation (Fox et al., 2018; Kesharwani et al., 2015a). The high constancy in the structure of dendrimers is mainly due to the controlled repetitive synthesis technique and number of functional groups present in the intermediate and the peripheral layers (Patri et al., 2005). Major classes of dendrimers that have been synthesized and used as drug carriers are polyamidoamine (PAMAM), PPI, poly-L-lysine, melamine, poly(etherhydroxylamine), poly(ester amine), polyglycerol, poly(2,2-bis (hydroxymethyl) propionic acid), dimethylolpropionic acid dendrimers, and aminobis (methylene phosphonic acid) scaffold dendrimer (Madaan et al., 2014).

Over the past few years, dendrimers have been center for research since it can produce personalized medicine and attain controlled drug delivery (Sherje et al., 2018). A typical dendrimer consists mainly of four parts: (1) initiator core having one or two more reactive groups to which the dendrons are attached, (2) interior layers or shell comprising of the repeating branched units bound to the initiator core where each layer is a generation, (3) terminal functional group present in the extremities of the nanostructure which determines the nature and the drug entrapping ability of the dendrimer, and (4) void spaces (Fig. 8.1) (Sherje et al., 2018).

The type of interior core:

1. determines the number and size of dendron branches formed,
2. determines the number of cavities formed, and
3. enables host and guest interaction and promotes encapsulation of the guest molecule.

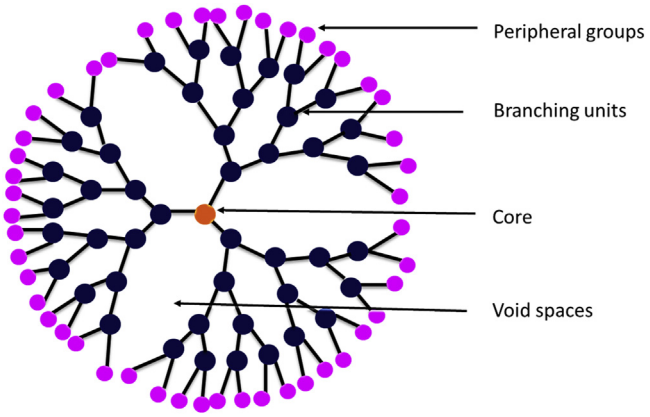

FIGURE 8.1 Structure indicating the components of a dendrimer unit.

The drug targeting ability of the dendrimers is due to the attachment of different functional groups, therapeutic agents, imaging agents in a controllable manner at its terminal position (Patri et al., 2005; Tekade et al., 2008b). Its monodispersed nature, three-dimensional (3D) structure, and high functionality make it an excellent carrier for drugs, gene delivery (Fox et al., 2018), improves the residence time of the drug, and increases its stability. Dendrimers exist primarily in two shapes: (1) ellipsoid and (2) spherical shape. Sometimes the initiator component determines the shape of the formed dendrimer such as the dendrimers developed by ethylenediamine (EDA) have an ellipsoid shape (Yang and Kao, 2006). The shape of the dendrimer determines the binding of the functional group on the dendrimer surface and interior of the dendritic structure.

A three- or four-branched unit is added to the core to form the first generation, followed by the addition of two-branched units to each monomer of the previous generation producing the second generation of the dendrimers. Repetitive additions of branched units are done so as to produce the desired generation.

Low-generation dendrimers (0, 1, 2) are highly branched and are asymmetric in nature having open amorphous structure, whereas high-generation dendrimers (4 or higher up to 12) are globular in nature and can integrate a larger amount of hydrophobic drug molecules in their cavities as well as can carry several drug molecules on their surface (Fig. 8.2) (Selin et al., 2016; D'emanuele and Attwood, 2005; Choudhary et al., 2017). This happens owing to the increase in dendrimer generation, which also increased the number of voids within the dendrimers that facilitate the solubilization of drug at a large extent (Choudhary et al., 2017; Jain et al., 2015). They are densely packed in the outer periphery to form a membrane-like structure. The core, interior shells, and the periphery affect the physical and chemical properties of the dendrimers.

The shape and structure of the dendrimer, the number of dendrons radiating, and the number of cavities formed are manipulated by the core material used. Several initiator cores are used, which include phosphorus, nitrogen, as well as various branching units (Fox et al., 2018). The nature of the end group determines the solubility and reactivity of the dendrimers, that is, dendrimers possessing hydrophilic terminal groups will be soluble in the polar solvent and vice versa. The property of having a hydrophobic interior and

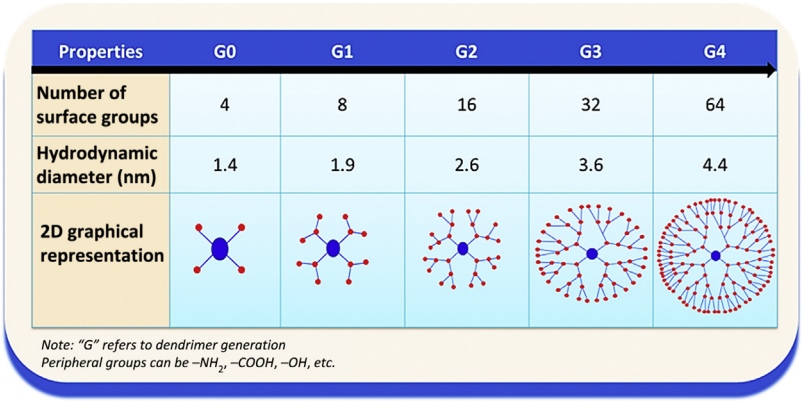

FIGURE 8.2 Graphical representation of dendrimers with different generations and its approximate diameter.

hydrophilic exterior permits its use as a solubility enhancer. This happens due to the entrapment of the bioactive molecule within the hydrophobic void and subsequent interaction with the tertiary amine or amide through electrostatic and hydrogen bonding, which enhances solubility. The drug can also be entrapped within the dendrimer physically (D'emanuele and Attwood, 2005).

Linking of the drug on the surface of the dendrimer through hydrogen and covalent bonding in hydrolyzable and biodegradable linkages is responsible for the controlled release of the drug (D'emanuele and Attwood, 2005). In this regard the shape, size, and surface charge is modified, so that the dendrimers can circumvent cellular membranes (Mukherjee et al., 2010), interact with deoxyribonucleic acid (DNA) molecules (Navarro and Tros de Ilarduya, 2009), and solubilize hydrophobic drugs (Fox et al., 2018; Markatou et al., 2007).

The molecular weight of the dendrimers is affected by the core, branch cell multiplicity, the generation, which controls the size of the dendrimers that are in turn modulated by the synthesis method (Ghaffari et al., 2018). The type of the molecules used to make the branches, the flexibility of the bonds within the dendrimers, and the nature of the surface groups determines the internal structure of the dendrimer (D'emanuele and Attwood, 2005). It has been found that polyionic dendrimers do not adhere to their fixed shape and may undergo variation in size, shape, and flexibility as the generation increases (Abbasi et al., 2014). Several theories have suggested that the dendrimers constitute of flexible bonds showing maximum density at the center and least density at the periphery, which was confirmed by the small-angle neutron scattering and small-angle X-ray scattering (D'emanuele and Attwood, 2005). Dendrimers inherit multivalent characteristics that indicate the reactivity and multiinteractive nature of the terminal groups with other molecules.

When dendrimers are being used for therapeutic purposes, the drug is being conjugated to the dendrimer via cleavable linkers such as amides, esters, hydrazones, which is later activated by external stimuli such as magnetic field, light, oxidation, and change in the surrounding pH or via enzymes. These drug–dendrimer conjugates offer the advantage of fewer side effects, better therapeutic efficacy.

8.1.1 Specific properties of dendrimers

Some important properties of dendrimers are given in Figs. 8.3 and 8.4 are and detailed below:

1. Dendrimers made up of small molecules or containing PEG or dendrimers having PEGylated surface show low immunogenicity (Chen et al., 2004).
2. As the molecular mass of the dendrimers increases, their viscosity goes on decreasing.
3. Dendrimers can be used as photo suitable hosts. Photochemical modification of the peripheral groups of the dendrimers can control the encapsulation and the liberation of the guest molecules.
4. Dendrimers can act as highly efficient light-harvesting antennae.
5. The amine-terminated dendrimers with a full generation (i.e., G1.0, G2.0, and G3.0) show higher intrinsic viscosity as compared to ester terminated half generation (i.e., G1.5, G2.5, G3.5, and G4.5). This might be owing to the intermolecular hydrogen bonding.
6. The dendrimers can incorporate a wide range of molecules such as drugs, metals, imaging substances, and can help in reducing drug toxicity and increases the residence time in the system. Thus it facilitates the controlled release of the drug. In addition, it has also been reported to have antiinflammatory, anti-HIV (human immunodeficiency virus), antiarthritis property (Gorkhe et al., 2018).
7. The monodisperse nature of the dendrimers improves its bioavailability and biodistribution.

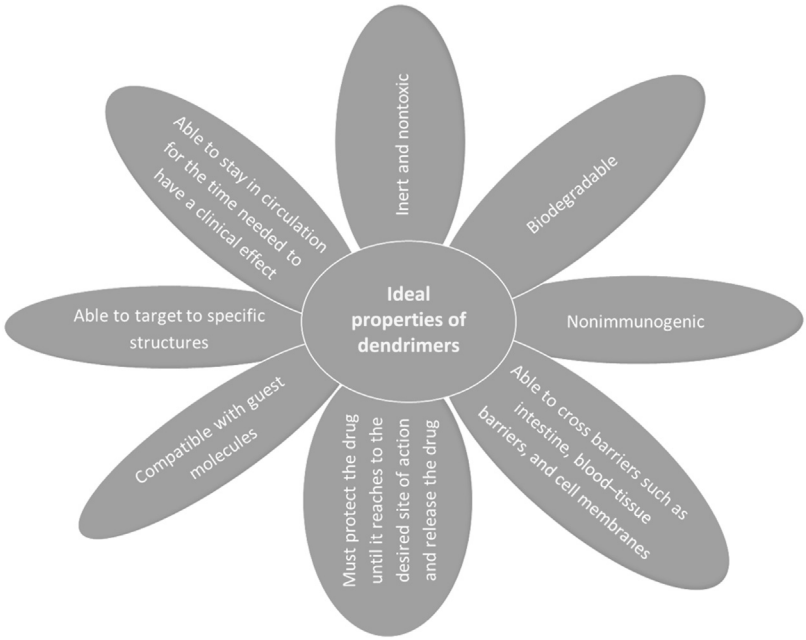

FIGURE 8.3 Ideal properties of dendrimers.

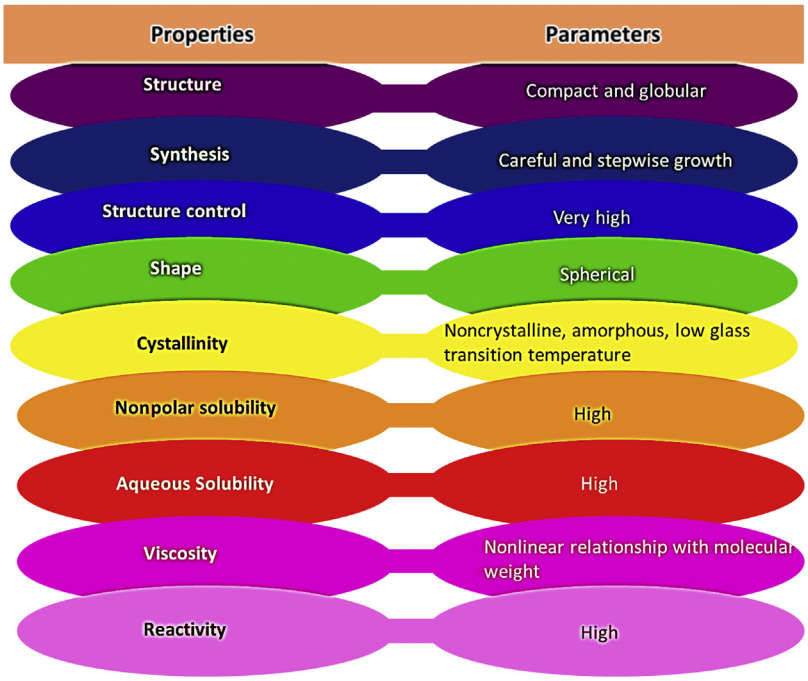

FIGURE 8.4 General properties of dendrimers.

8. The dendritic polyester shows higher solubility than tetrahydrofuran (THF) (Gorkhe et al., 2018).
9. Dendrimers are termed "artificial protein" due to their structural resemblance with other biological components. For instance, G7.0 and G10.0 dendrimers imitate the histone protein, PAMAM G3.0 (3.1 nm in diameter) resembles insulin, G4.0 (4 nm) resembles cytochrome C, G5.0 (5.3 nm) resembles hemoglobin (D'emanuele and Attwood, 2005), and G6.0 (6.7 nm) resembles prealbumin, G7.0(8.2 nm) resembles hemerythrin by shape and size (Fig. 8.5) (Choudhary et al., 2017).
10. The polarity, ionic strength, and pH of the solvent affect the structure of the dendrimers to be either compact or open, for example, PPI and PAMAM dendrimer having terminal NH_2 show open conformation at low pH owing to charge repulsion in between protonated tertiary amines in the interior and the primary amines at the periphery. It exhibits back-folding neutral and higher pH (pH $>$ 9) due to hydrogen bonding formed between the positively charged amine groups present at the periphery and the interior uncharged tertiary amine group thus showing a compact core.
11. It was found that the positively charged dendrimers were more toxic than the negatively charged (Chen et al., 2004; Choudhary et al., 2017), PEGylated, or neutral dendrimers. These positively charged dendrimers interact with the negatively charged cell membrane thus reducing cell integrity and increasing permeability. This, in turn, causes leakage of cytosolic proteins such as lactate dehydrogenase (LDH) and luciferase and finally membrane disruption and cell lysis (Jain et al., 2010). Therefore surface modification is carried out using negatively charged molecules (e.g., carboxyl,

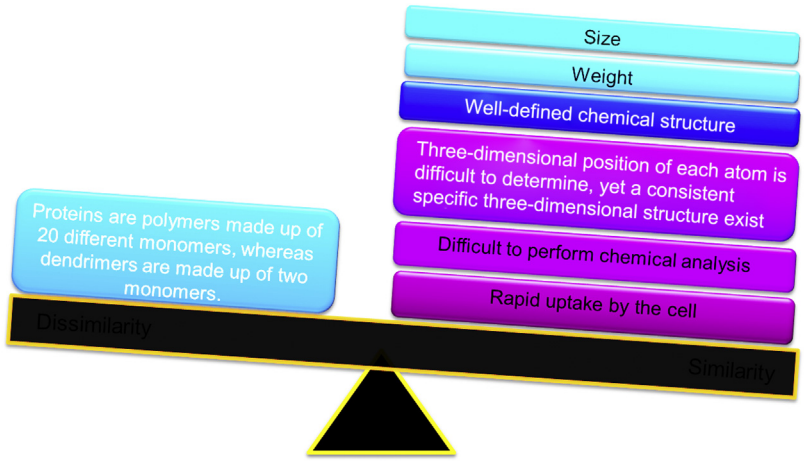

FIGURE 8.5 Comparison of protein versus dendrimers.

hydroxyl, and acetyl groups) to reduce the association between the cationic dendrimer and anionic cell membrane (Pearson et al., 2014). Thus the molecules, namely, pyrrolidone, carboxybetaine, sulfobetaine, triazolium carboxylate, phosphorylcholine are considered nontoxic for surface attachment to the dendrimers (Svenningsen et al., 2016).

12. Moreover, membrane disruption is elevated with an increase in the charge densities on the dendrimers and an increase in the concentration of spheroid dendrimers as well as linear polymers. Further, the membrane disrupting ability and pore formation is increased with the rise in the molecular size (Jain et al., 2010).
13. In order to target certain cells, dendrimers are conjugated to specific ligands on their surfaces. For instance, G5.0 PAMAM dendrimers were conjugated to folic acid (FA) to increase its uptake by the cells and to improve the delivery of the attached methotrexate via both in vivo and in vitro.
14. Dendrimers exhibit different toxicities such as in vitro cytotoxicity, hemolytic toxicity, and in vivo toxicity. This type of toxicity is mainly exhibited due to the positive charge present on the dendrimeric surface which interacts with the negative charge on the cell membrane. This results in an increase in white blood cells count and fall in red blood cells (RBCs) count (Asthana et al., 2005).
15. Toxicity of the dendrimers is mainly influenced by its generation (i.e., size), amount and type of the terminal groups present on the surface, incubation time. Lower generation dendrimers are better than higher generations in terms of cytotoxic, immunogenicity, and biocompatibility (Duncan and Izzo, 2005).
16. In fact, dendrimers undergo rapid renal clearance, though they get accumulated in the liver and kidney owing to the small size and positive charge.
17. Dendrimers can circumvent the uptake of nonspecific reticuloendothelial cells due to nanoscopic size (Gorkhe et al., 2018). The nanosize of the dendrimers enables it to passively target tumor tissues as they can accumulate much more in tumor cells than in the normal cells. This phenomenon is termed as enhanced permeability and retention (EPR) effect.

18. In contrast to other drug-delivery approaches such as carbon nanotubes, traditional polymers, dendrimers are highly nonspecific and have limited diversity (Abbasi et al., 2014).
19. The solubilizing ability of the dendrimers is affected by the (Choudhary et al., 2017):
    a. type of dendritic core,
    b. the concentration of the dendrimer in the solution,
    c. the pH of the solution,
    d. dendrimer generation,
    e. nature of dendrimer surface,
    f. presence of salts, and
    g. temperature.

A rise in the loading efficiency of flurbiprofen in water using PAMAM dendrimer (G4.0) was seen where maximum loading was at pH 10, followed by at pH = 7 and least at pH = 2 (Asthana et al., 2005). This is mainly due to the modulation of their ionization and surface interaction with a change in pH (Choudhary et al., 2017). Rational selection of the dendritic core allows larger interior cavities that enable improvement of hydrophilicity of hydrophobic groups. The solubility of the drugs increases linearly with increase in dendrimers concentration (Prajapati et al., 2009). High salt concentration affects conformational changes in the dendrimers with a high amount of back-folding. Furthermore, at a lower concentration of salt, the unfolding of the dendritic structure occurs owing to the repulsion in between ionized groups of the dendritic structure (Choudhary et al., 2017; Gupta et al., 2007). The heating effect on solubilization of ibuprofen in G4.0 PAMAM dendrimers at various temperatures was also investigated. Drug solubility tends to increase with the rise in temperature (Milhem et al., 2000). However, the effect of temperature on the solubilizing ability of the dendrimer is yet to be explored (Jain and Tekade, 2013).

8.1.2 Disadvantages

1. Dendrimers show toxicity owing to the presence of a peripheral amine, guanidine, carboxylate, sulfonate, or phosphonate group (Malik et al., 2000a; Chen et al., 2004).
2. Fast clearance from system circulation.
3. Nonspecific drug delivery.
4. Due to the open network of the dendrimer, they show poor control on drug release.
5. In a covalently bound drug–dendrimer complex, there is an abrupt release of drug from the drug–dendrimer structure after exposure to the biological fluids due to lack of interactive forces with the structure (Choudhary et al., 2017).

8.1.3 Synthesis of dendrimers

The synthesis of dendrimers was first reported by Tomalia et al. in 1985. There are several methods for the preparation of dendrimers (Fig. 8.6), few of them are as follows:

1. divergent growth method;
2. convergent growth method;

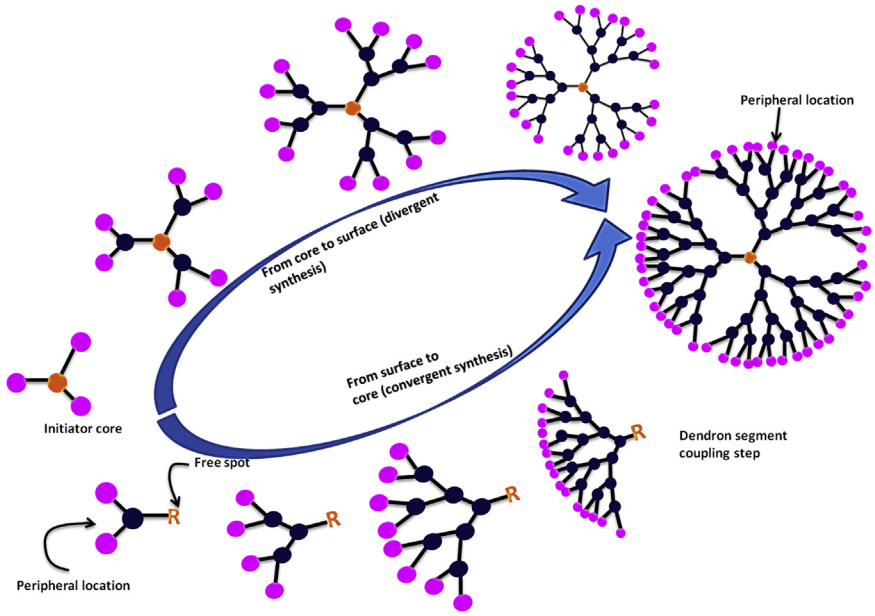

FIGURE 8.6 Schematic diagram of dendrimer synthesis.

3. "hypercores" and "branched monomers" growth;
4. "double exponential" growth;
5. "lego" chemistry; and
6. "click" chemistry.

Out of these methods, most popularly used techniques are divergent growth method and convergent growth method.

8.1.3.1 Divergent growth method

This method was used the first time for the preparation of dendrimer by Tomalia et al. where they used EDA and ammonia initiator cores as the starting material. The interior layers were prepared using *N*-(2-aminoethyl) acrylamide (Tomalia et al., 1985).

It required the Michael addition of methyl acrylate to an appropriate amine initiator core followed by amidation of the esters with excessive EDA. A concept called de Gennes dense-packing effect states that the dendritic molecules grow in size with the further addition of branching units, so that an upper generation limit is reached in which end groups on the globular surface becomes packed impenetrably. For instance the highest generation limit for ethylene diamine–cored PAMAM dendrimers is 10 (Yang and Kao, 2006). As reported by Tomalia et al. (1985), the yield for PAMAM dendrimer was reported to be 98%–100%. This method produced a lot of structural defects owing to incomplete reaction of groups which can be counteracted by excessive adding of monomer units (Selin et al., 2016).

8.1.3.2 Convergent growth method

The convergent method of dendrimer preparation is opposite to that of the divergent method. In the former approach sometimes, it is difficult to work with a single molecule; therefore the preparation of the dendrimer is done by starting at the periphery and working toward the core by attaching the surface units with more monomers. When enough growth of the branching units is attained then several such units are further attached to a single-core compound. This method offers some advantages such as it requires cheap reagents, fast synthesis, exponential growth, and formation of large-sized dendrimers. This method also faces hindrances, that is, purification of the contaminated product, low yield while producing large structures; however, only low-generation dendrimers can be produced owing to the steric hinderances seen while attaching the dendrons to the core (Nanjwade et al., 2009).

8.1.3.3 "Hypercores" and "branched monomers" growth

This method uses the advantages of both methods, that is, convergent and divergent techniques. It requires assembling of the oligomers before they are joined to give dendrimers in high yield (Nanjwade et al., 2009).

8.1.3.4 "Double exponential" growth

This technique was first studied by Shinkai, Fréchet, and Roy. In this technique the number of repeat units per dendrimer is produced according to a double exponential function when generation is n. It uses a single trifunctional monomer having an orthogonally protected functional group (Kawaguchi et al., 1995).

8.1.3.5 "Lego" chemistry

In this method phosphorus dendrimers are prepared using highly functional monomers and functional groups. Terminal groups are made up of phosphines and hydrazines. This method also possesses some advantages such as it requires the least volume of solvent to produce, allows easy purification, and produce environmentally friendly by-products such as water and nitrogen (Nanjwade et al., 2009).

8.1.3.6 Click chemistry

This method was first proposed by Kolb and Sharpless (2003) who joined smaller units using heteroatoms. This method produces dendrimers with different peripheral groups in pure form with a good yield (Nanjwade et al., 2009).

8.2 Classes of dendrimer

The growth in the development of dendrimer-based novel carrier is rapid that might be owing to the recent innovation, especially in synthetic chemistry and evaluation techniques. In addition a series of dendritic scaffolds are available that have not the only definite size in the nanoscopic domain but also holds plenty of functional end groups. Dendrimers can be classified as different classes as shown in Fig. 8.7.

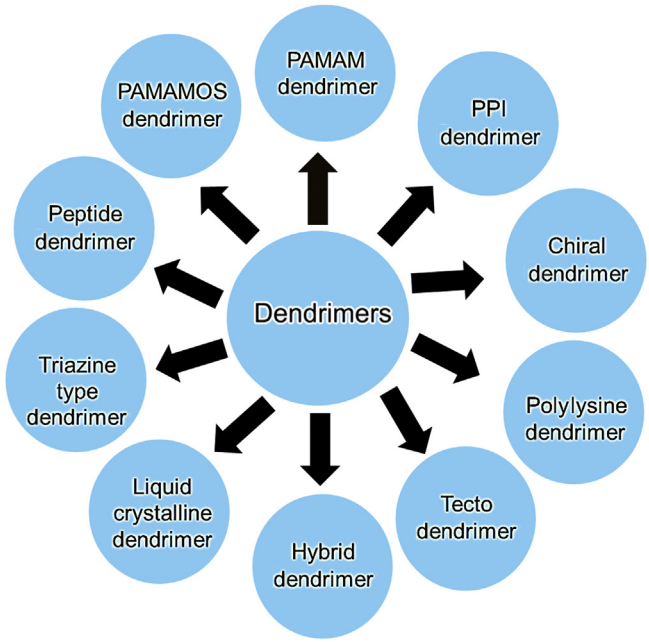

FIGURE 8.7 Different classes of dendrimers.

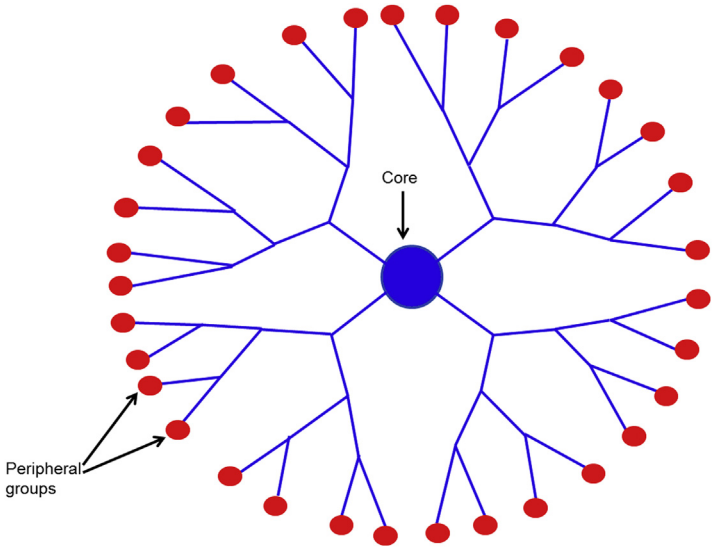

FIGURE 8.8 Structure of PAMAM dendrimer. *PAMAM*, Polyamidoamine.

8.2.1 Polyamidoamine dendrimer

PAMAM dendrimer was first reported by Tomalia et al. in 1980 and was the first class of dendrimer to be synthesized and commercialized. They are highly branched synthetic macromolecules possessing defined structure and composition (Fig. 8.8). Since PAMAM dendrimers are synthesized in a controlled manner, they come with an advantage of low

polydispersity. They have a wide range of biomedical application due to their tunable properties. The core in PAMAM dendrimer generally consists of EDA, ammonia (NH_3), cystamine, etc. Further, the repeating unit of methacrylate and EDA is added to the core as per the generation required for dendrimer (G1.0, G2.0, G3.0, etc.). G0.5 generation with terminal carboxylate groups can also be synthesized by terminating the reaction after addition of methacrylate (Fox et al., 2018). The superficial branches of the PAMAM may have different functional groups such as amines (NH_2), hydroxyl (OH), carboxymethyl ($COOCH_3$), tert-butyloxycarbonyl (Boc), aldehyde (CHO), methyl (CH_3), or sodium carboxy (COONa). However, the NH_2 group in dendrimer may help in delivering genetic material to the cell (Abedi-Gaballu et al., 2018).

The most common ways used for functionalization of terminal amine group include hydroxyl, carboxyl, or conjugation with hydrocarbon, or PEG chains. The internal cavity and the peripheral functional groups can be modified for the encapsulation of drug, genes, or an imaging system. Their size range is from 1 to 14 nm correspondingly from generation G0.0 to G10.0. A full-generation PAMAM dendrimer with a primary amine group exhibits a p$Ka \approx 6.85$ at the surface, while dendrimer with a tertiary amine group in the core has p$Ka \approx 3.86$. In addition, certain generations of PAMAM dendrimers are also able to penetrate the gastrointestinal wall by overcoming the epithelial barrier and thus can offer the advantage of oral delivery (Sadekar and Ghandehari, 2012).

The core, as well as the peripheral groups in the PAMAM, can be functionalized to obtain various properties such as for oral delivery (Raval et al., 2019), for gene delivery, to overcome the epithelial barrier, for improving encapsulation efficiency.

Electron micrographs of the PAMAM dendrimers showed that they possess hollow cores. Further, fourth-, fifth-, and sixth-generation dendrimers have a topology similar to the micelles and can be used for encapsulation of small molecules. The lower generation dendrimer G3.0 possess open and flexible structure while higher generations G7.0−G10.0 possess rigid structure due to steric branch crowding; hence, these generations have less encapsulation efficiency (Fox et al., 2018). However, the flexibility of PAMAM dendrimers decreases with the increase in the generation. The core size as well as the congestion at the surface affects encapsulation and release profile. Moreover, various compounds can be conjugated with the PAMAM dendrimers to improve these properties, for instance, tris (hydroxymethyl) aminomethane is used for antibacterial compounds. For cell-specific targeting the amine groups can also be conjugated with folates or carbohydrates (i.e., glycodendrimers) (Fox et al., 2018; Dhakad et al., 2013; Kesharwani et al., 2011b).

Lower generation PAMAM dendrimers with terminal amine group have been found to increase the endocytosis of DNA in the nucleus. This might be owing to the electrostatic attraction between the positively charged terminal amino group of the dendrimer and the negatively charged phosphate group of the DNA molecules (Fig. 8.9). Further, the transfection ability is dependent on the DNA−dendrimer ratio as well as the generation of the dendrimer. Sugar molecules such as cyclodextrins or hydrophobic molecules such as Oregon Green 488 dye have been found to increase transfection (Abedi-Gaballu et al., 2018). PAMAM due to the presence of a positively charged terminal amino group can form complexes with DNA and can act as a potential carrier for gene delivery. In addition, lower generations have also been found to have more endocytosis capacity.

For the controlled drug release through PAMAM dendrimer, drugs with low solubility profile (i.e., hydrophobic drugs) are to be physically encapsulated or entrapped inside the

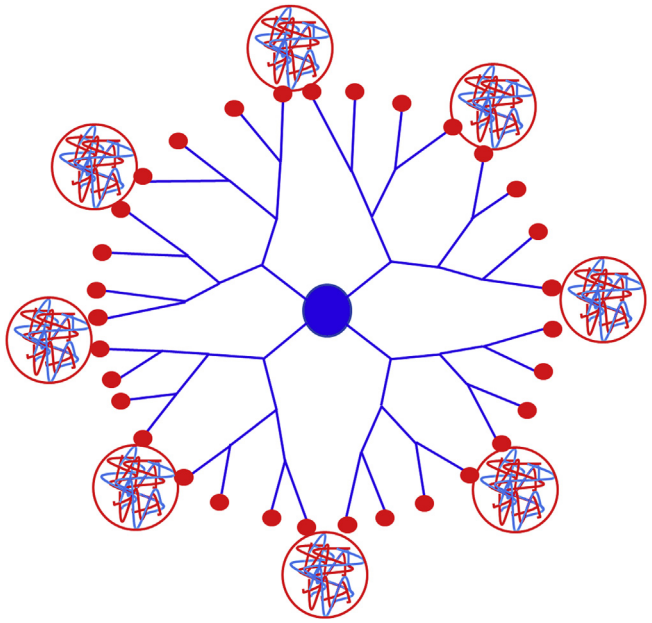

FIGURE 8.9 DNA complexes with PAMAM dendrimers (dendriplexes). *DNA*, Deoxyribonucleic acid; *PAMAM*, polyamidoamine.

void space. This leads to improvement in the aqueous solubility. The major physical interactions that involved are covalent bonding, hydrogen bonding, and electrostatic interaction (Fig. 8.10). For the drug release from the PAMAM dendrimer, lower pH is favorable because the amine groups on the periphery will remain protonated and their confirmation will be altered, which will trigger drug release. This property of PAMAM dendrimer makes them useful for delivery of anticancer drugs since the microenvironment of the tumor is acidic. Thus we can say that the PAMAM dendrimer has a pH-dependent property of drug release, which increases with a decrease in the pH (Abedi-Gaballu et al., 2018).

PAMAM dendrimer/carbon dot (CD) nanohybrid can be used for dual drug loading to eliminate the problem of multidrug resistance (Fig. 8.11). Blue emitting CDs can be first synthesized and can be covalently linked to doxorubicin (DOX) forming CDs/DOX complex, which can be further linked with PAMAM dendrimer containing a targeting ligand and efflux inhibitor. The example of targeting ligand which can be used is arginine–glycine–aspartic acid (RGD) peptide and the efflux inhibitor can be D-α-tocopheryl PEG 1000 succinate (TGPS). Here in this system the CDs can provide fluorescence imaging by luminescence, TGPS decreases the ATP levels by inhibiting the P-glycoprotein and increasing the reactive oxygen species (ROS) production and RGD provides targeting to the cancer cells. Hence, this type of system has a potential application in theranostics (Li et al., 2019).

The PAMAM dendrimer can be coupled with more than one drug along with the targeting moiety and linker to attach with the receptors present on the target cells. Thus a single approach can provide multiple applicability. PAMAM dendrimers possess cytotoxicity profile which is dependent either on their generation, concentration, or the surface

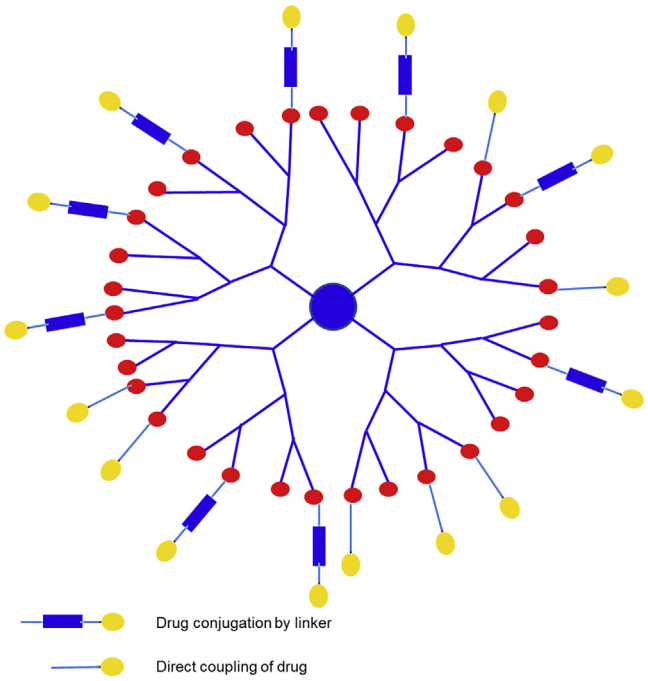

FIGURE 8.10 Drug conjugation with PAMAM dendrimer either directly or with the help of a linker. *PAMAM*, Polyamidoamine.

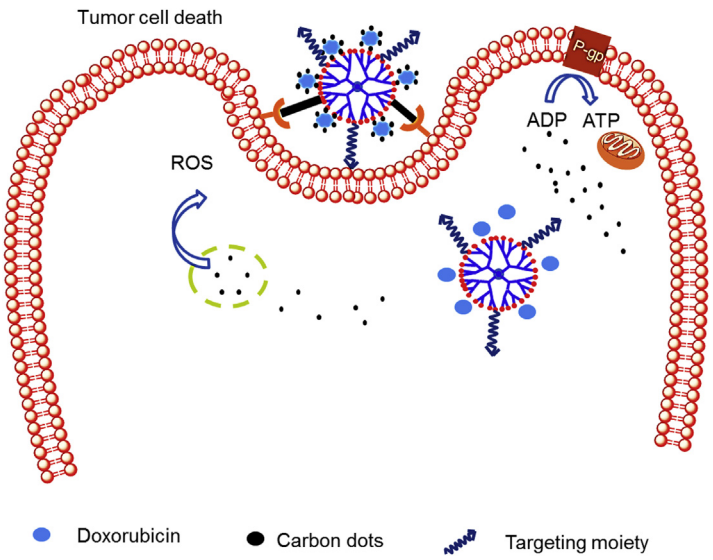

FIGURE 8.11 Dual drug loading in PAMAM dendrimer to avoid the problem of multidrug resistance. *PAMAM*, Polyamidoamine.

charge present on the dendrimer (Kesharwani et al., 2011a). Generally, the amine-terminated systems possess the highest cytotoxicity followed by carboxyl terminated and then the hydroxyl terminated with comparatively less cytotoxicity. The toxicity profile of the PAMAM dendrimer can be studied by LDH assay (Cui et al., 2018).

8.2.2 Glycodendrimers

Glycodendrimers indicate the presence of sugar moiety along with the dendrimers structure. They can be categorized into three main types based on the location of the sugar molecule either as the core, as peripheral functional groups, or in the branching units (Fig. 8.12). Hence, they are mainly of three types, that is, carbohydrate centered, carbohydrate coated, and fully carbohydrate based (Turnbull and Stoddart, 2002). Glycodendrimers contain sugar molecule in their structure which may be present as core molecule, as a peripheral group or entirely throughout the branches.

The most common method used for the preparation of glycodendrimers is a modification in the commercially available dendrimers. It produces glycodendrimers with low polydispersity. The divergent approach for synthesis is more preferred on a commercial basis, but it offers a disadvantage of having more number of reaction steps while convergent method solves this problem as well as easier isolation of product (Turnbull and Stoddart, 2002). The most common method to prepare glycodendrimer is by modifying the existing dendrimer, but the divergent approach is preferred to produce dendrimers with high polydispersity. The construction of the glycodendrimers involves three main steps. The first is the synthesis of the dendritic wedges which contains carbohydrates as a structural unit. The second step involves the formation of a linkage between these synthesized wedges with the branching components. Eventually, the final step involves the attachment of these dendrons to the core. The convergent synthesis in case of glycodendrimers has a disadvantage that it requires larger quantities of carbohydrate to be displayed; the peripheral carbohydrate groups need to be protected during the synthesis procedure; hence, it requires protection and deprotection steps which may result in steric crowding resulting in a decrease in coupling efficiencies (Turnbull and Stoddart, 2002).

It has been found that maltose containing neutral and positively charged fourth-generation glycodendrimers possess immunostimulatory properties on human dendritic cells in HIV infection (Córdoba et al., 2013). Maltose containing fourth-generation dendrimer has been found to show immunostimulatory action on dendritic cells in HIV infection.

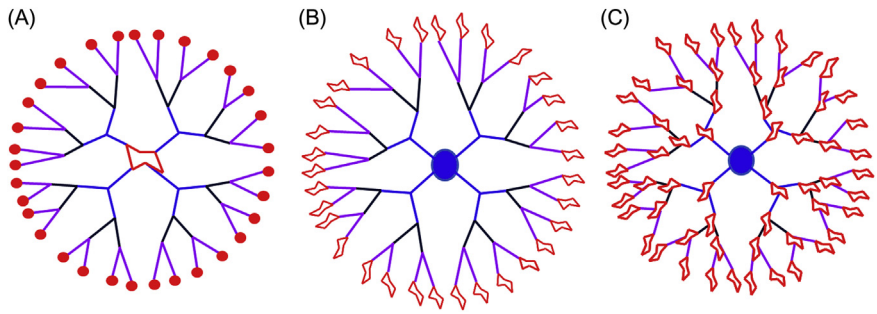

FIGURE 8.12 (A) Carbohydrate present as core in the center, (B) carbohydrate as peripheral groups, and (C) fully carbohydrate based.

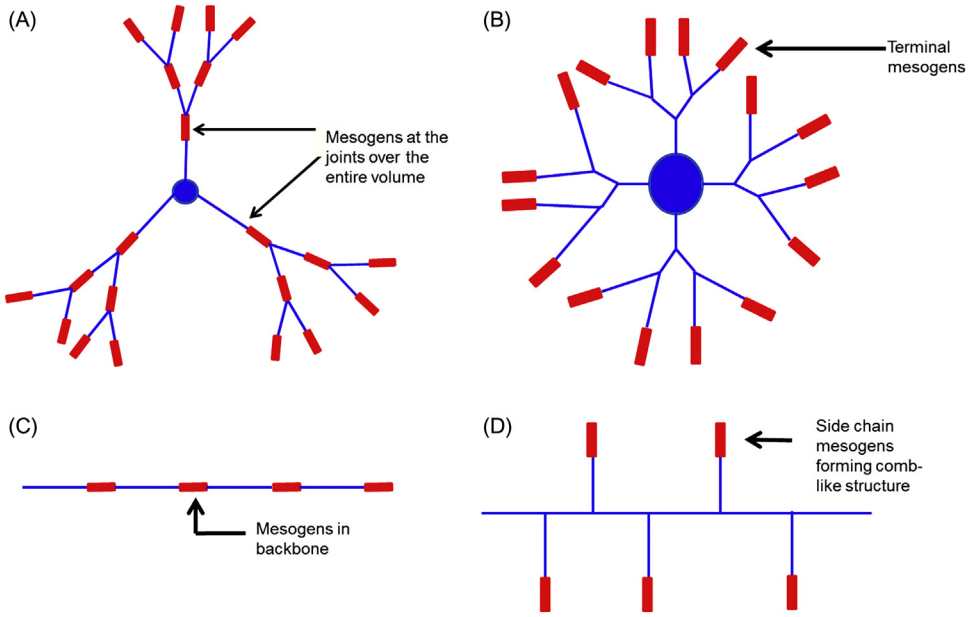

FIGURE 8.13 (A) Mesogen in joints over the entire volume, (B) dendrimer with terminal mesogens, (C) mesogen in backbone, and (D) dendrimers with side chain mesogens having a comb-like structure.

8.2.3 Liquid crystalline dendrimers

Liquid crystalline (LC) dendrimers apart from regular hyperbranched dendritic structure possess certain additional structural units known as mesogens or mesogenic groups capable of forming mesophases. Mesogens are the rigid rod-like structure with strong orientational interactions providing a gain in enthalpy due to the formation of anisotropic mesophases. LC dendrimers are those which contain rigid rod-like mesogens capable of forming mesophases. The main three structural elements of an LC dendrimer are a mesogenic group, an aliphatic spacer, and a polymer chain. The spacers are essentially required for the formation of LC mesophase. There may be variation in the position of attachment of the mesogenic groups as shown in Fig. 8.13 (Ponomarenko et al., 2001).

In the formation of the LC dendrimer, it is important to know whether the dendrimer will delay the LC properties of mesogens or high concentration of the mesogens will lead to certain preorganisation changes to the formation of mesophases. For instance, PPI−LC dendrimers exist with different mesogens (e.g., pentyloxy and decyloxycyanobiphenyl at the periphery) and the spacer (e.g., a pentyl group) in both the types (Fig. 8.14) (Guillon and Deschenaux, 2002).

8.2.4 Peptide dendrimers

Peptide dendrimers possess either a peptidyl-branching core or peripheral peptide chains or both. In a broader sense, they can be defined as dendrimer containing peptide

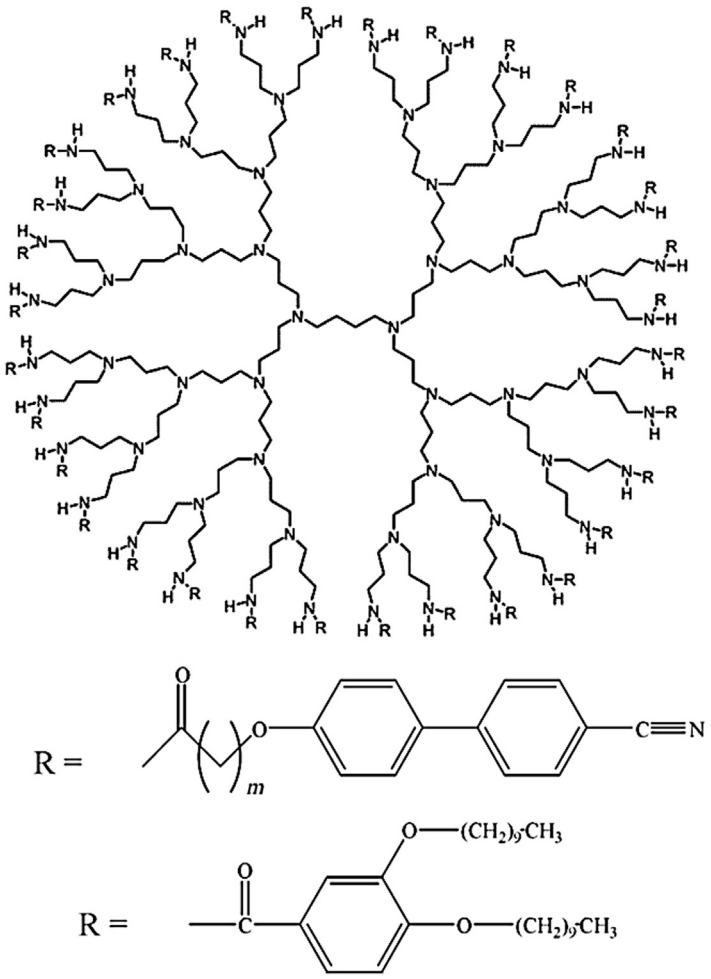

FIGURE 8.14 PPI dendrimer with two different types of mesogens. *PPI*, Poly(propylene imine). Source*: Adapted with permission from Pedziwiatr-Werbicka, E., Ferenc, M., Zaborski, M., Gabara, B., Klajnert, B., Bryszewska, M., 2011. Characterization of complexes formed by polypropylene imine dendrimers and anti-HIV oligonucleotides. Colloids Surf. B: Biointerfaces 83 (2), 360–366.*

bonds. Peptide dendrimers are generally used as molecules that mimic proteins and are employed in nonviral vaccine delivery, as an immunogen, and a diagnostic agent. Peptide dendrimer offers several advantages that include the formation of nontoxic metabolites, economic for bulk synthesis, and easy purification (Manikkath et al., 2017). Peptide dendrimers contain peptide bonds in their structure, thus mimic protein structure and are preferred for their biocompatible properties. The molecular weight of the peptide dendrimers ranges from 2 to 100 kDa, which may contain large protein structures. It generally depends on the generation of the dendrimer as well as the protein/peptide attached to the terminal functional group of the dendrimer. Peptide dendrimers are classified into three major types that include grafted peptide dendrimers, which contain unnatural amino acids or organic moieties as the core and proteins, or peptides are attached to it as peripheral functional groups.

The other type contains natural amino acid as core and is known as essentially branching polyamino acids. The core of this type of peptide dendrimer is a natural amino acid. The third type mostly contains conventional peptides. Multiple antigenic peptides (MAP) are a part of this group as it possesses many biological and biochemical applications. MAP contains amino acids as a core molecule while peptidyl chains as a peripheral functional group (Fig. 8.15) (Sadler and Tam, 2002).

Peptide dendrimers are now studied as a potential carrier for nonviral vector for delivery of genes. Peptide dendrimers possess certain characteristics that make them suitable as a vector for gene delivery such as flexibility, amino acid groups on the external surface which provides them with properties similar to proteins like biocompatibility and water solubility. In addition, they are resistant to proteolytic degradation (Luo et al., 2012).

The gene transfection capability of a dendrimer can be increased by increasing the generation, but an increase in dendrimer generation results in steric hindrance that will create challenges in their synthesis. This problem can be overcome by the use of click chemistry technique in the synthesis of the dendrimer. Arginine has been found to increase transfection efficiency. Hence, it can be attached to the peripheral groups of peptide dendrimer. Apart from gene transfection, the peptide dendrimers have a major application in immunoassays. Small synthetic peptides act as an ideal antigen, but they lack the ability to bind

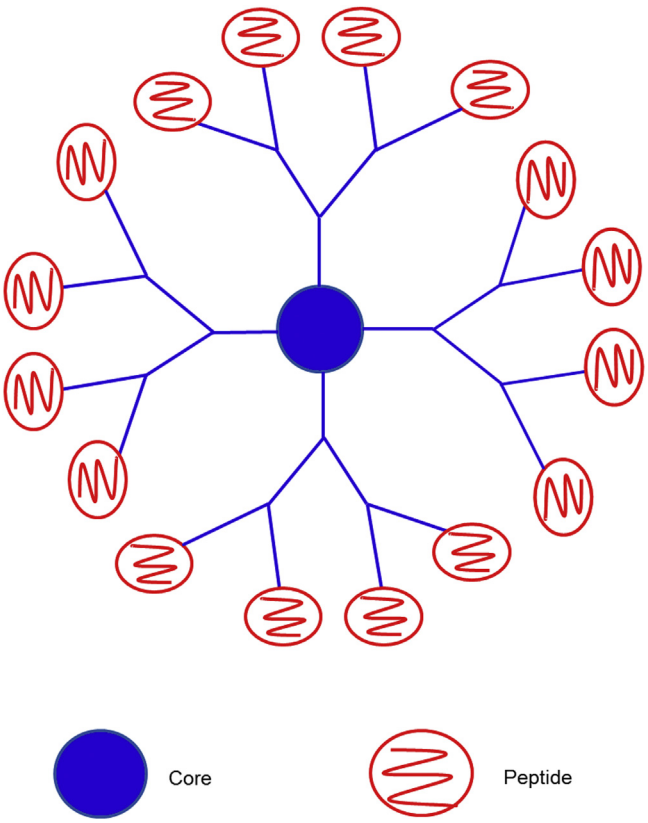

FIGURE 8.15 Peptide dendrimer.

solid surfaces. Peptide dendrimers can overcome this problem as they provide increased surface binding due to their multimeric nature (Luo et al., 2012). Peptide dendrimers have been used for their gene transfection property and in immunoassays. Certain molecules such as arginine, when attached to peptide dendrimers, increase the transfection ability.

8.2.5 Chiral dendrimers

Chiral dendrimers consist of three different parts in the structure such as the presence of stereogenic centers in the core of dendrimer, a branch containing stereogenic centers, attachment of the chiral molecules to the surface. These can be achieved by controlled synthesis of the dendrimer. The major areas in which chiral dendrimers are employed as a catalyst, in the separation of two enantiomers, in sensing, and in molecular recognition (Quintana et al., 2017).

Chiral dendrimers containing stereogenic centers are generally used as a catalyst, in the separation of enantiomers, and in molecular recognition. An example of the chiral dendrimer, which is studied is carbosilane dendrimers containing β cyclodextrins. It is used as a chiral stationary phase in capillary electrochromatography, a method required for separation of enantiomers. Other carbosilane dendrimers by modification with Levo-enantiomers of cysteine and *N*-acetyl-L-cysteine have been reported to possess chirality. Both the molecules used in the synthesis should be enantiomerically pure. Both the groups contain stereogenic centers as well as a thiol group, which can be used for attachment with the terminal olefin group through thiol-ene addition reaction (Quintana et al., 2017). The molecules used for the preparation of chiral dendrimers should be enantiomerically pure.

For instance, 1,1-binaphthyl molecules are used for the preparation of chiral dendrimers owing to its stable chiral configuration and asymmetric induction property (Fig. 8.16). Chiral dendrimers based on binaphthyl core are also used in selective enantiomer recognition of chiral compounds with the help of fluorescence. Use of fluorescence offers some advantages such as increased sensitivity, the capability to record real-time responses, and the possibility of detecting multiple modes. They can be used in high-throughput screening of chiral catalysts. Chiral dendrimers possess light-harvesting property that could amplify their fluorescence responses toward the chiral compounds (Pu, 2003).

8.2.6 Hybrid dendrimers

Hybrid dendrimers are those in which two dendritic segments, which are chemically different, are combined such that they are able to maintain the molecular branching structure independent of others. However, to achieve this is quite difficult. Hybrid dendrimers containing hydrophilic PAMAM dendrimer and hydrophobic organosilicon have been developed with a variety of applications. Another example of a hybrid dendrimer is triazine with phosphorhydrazone. The hybrid dendrimers possess inherited properties of individual dendrimer as well as some unique new properties. The arrangement of the two dendrimers in different ways such as a flexible core with a rigid shell or a flexible shell with a rigid core can lead to a better understanding of dendrimer performance. An additional advantage offered by hybrid dendrimers is that their production requires only a

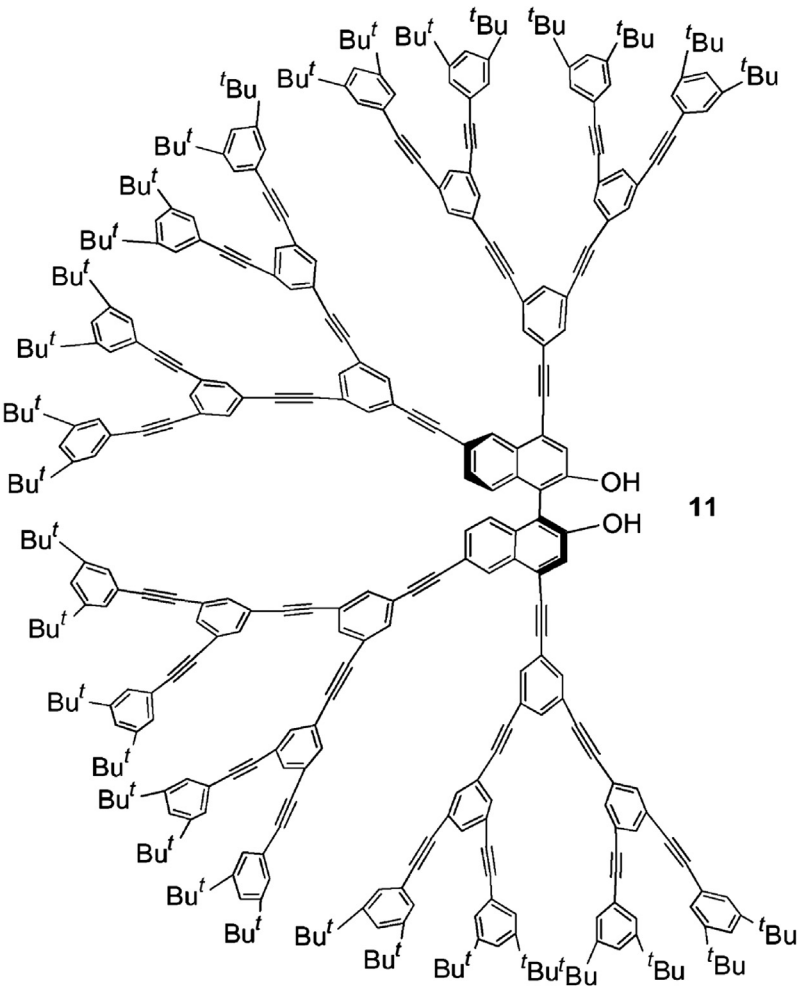

FIGURE 8.16 Chiral phenyleneethynylene dendrimer with binaphthyl core. Source: *Adapted with permission from Pu, L., 2003. Synthesis and study of binaphthyl-based chiral dendrimers. J. Photochem. Photobiol. A: Chem. 155 (1–3), 47–55.*

simple covalent linkage between two already formed dendrimers (Serkova et al., 2018). Hybrid dendrimers contain two chemically different molecules possessing new unique as well as inherited properties of the individual molecules.

An example of a hybrid dendrimer molecule with a considerable difference in nature is flexible carbosilane dendrimer with rigid polyphenylene dendrimer. The dendrimer can be synthesized in both the ways, that is, carbosilane as core and polyphenylene as a shell or else polyphenylene as core and carbosilane as a shell (Fig. 8.17) (Serkova et al., 2018).

8.2.7 Polyamidoamine organosilicon dendrimer

PAMMA organosilicon (PAMAMOS) is a type of a radially layered copolymeric dendrimer that contains PAMAM molecule as a hydrophilic core and organosilicon as peripheral groups (Fig. 8.18). They can be synthesized by Michael addition organosilicon moieties to

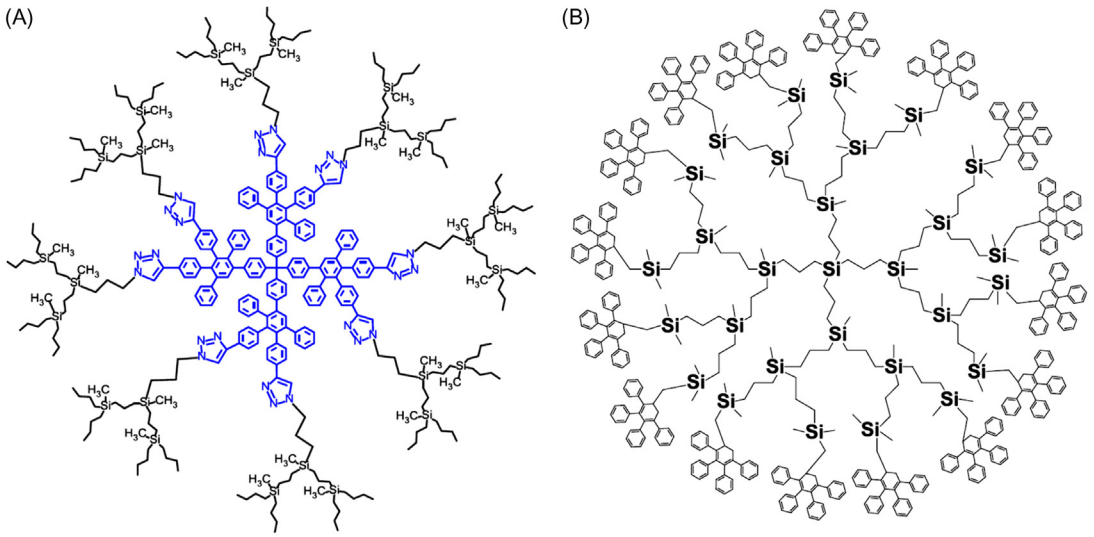

FIGURE 8.17 (A) Hybrid dendrimer with polyphenylene core and carbosilane shell and (B) hybrid dendrimer with carbosilane core and polyphenylene shell. Source: *Adapted with permission from Serkova, E.S., Krasnova, I.Y., Milenin, S.A., Selezneva, E.V., Tatarinova, E.A., Boldyrev, K.L., et al., 2018. Core/shell hybrid dendrimers: controllable rigidity determines molecular behaviour. Polymer 138, 83–91.*

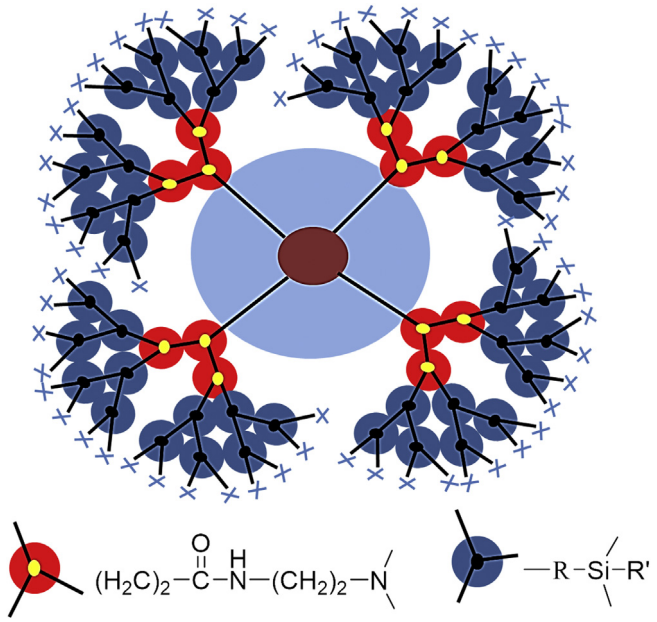

FIGURE 8.18 PAMAMOS dendrimer. *PAMAMOS,* Polyamidoamine organosilicon.

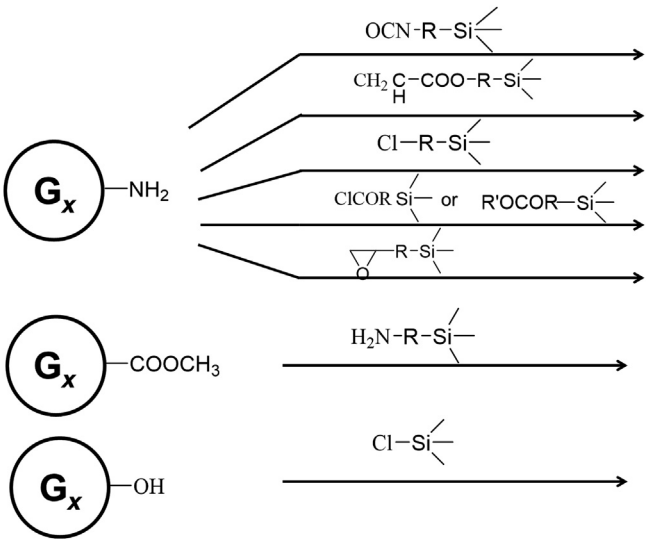

FIGURE 8.19 Synthesis of PAMAMOS dendrimer. *PAMAMOS*, Polyamidoamine organosilicon.

PAMAM dendrimer with an amine as terminal groups or by the process of haloalkylation of PAMAM dendrimer by chloroalkylsilicates or iodoalkylsilicates. They are also referred to as inverted micelle-type dendrimer containing highly branched amphiphilic molecules. Further, they can be used as organo-inorganic precursors in the preparation of other complex structures. PAMAMOS can be synthesized using inactive peripheral groups, namely, trimethylsilyl and trimethsiloxysilyl units and their reactive derivatives, for example, vinylsilyl, vinylsiloxylsilyl, and alkoxysilyl (Fig. 8.19) (Dvornic et al., 2000). PAMAMOS is a copolymeric dendrimer that contains hydrophilic PAMAM and organosilicon molecules.

For copolymeric dendrimer, it is essential that it has unique characteristics as well as features of the components. This characteristic feature of the copolymer was seen in solubility determination of the PAMAMOS dendrimer. The hydrophilic PAMAM dendrimers were observed soluble in water, methanol, and insoluble in hydrocarbons, while in case of hydrophobic trimethylsilyl (TMS) substituted PAMAMOS dendrimer, they were found insoluble in water and soluble in toluene. The partially substituted dendrimers were reported soluble in polar organic solvents, for example, chloroform and THF, while partially as well as fully substituted dendrimers retained the solubility in methanol, which is a characteristic of PAMAM. Generally, methyl siloxane polymers are not soluble in methanol, but this solubility feature of PAMAMOS is attributed to the unique structural geometry of dendrimers (Dvornic et al., 2000).

8.2.8 Core–shell tectodendrimers

The size of most of the dendrimers is large enough so that they can mimic protein structures, but still they are smaller than certain targets; hence it is necessary to synthesize dendrimers. But the size of dendrimer increases with the increase in the number of generations, which complicates the synthesis process. An important strategy that can be

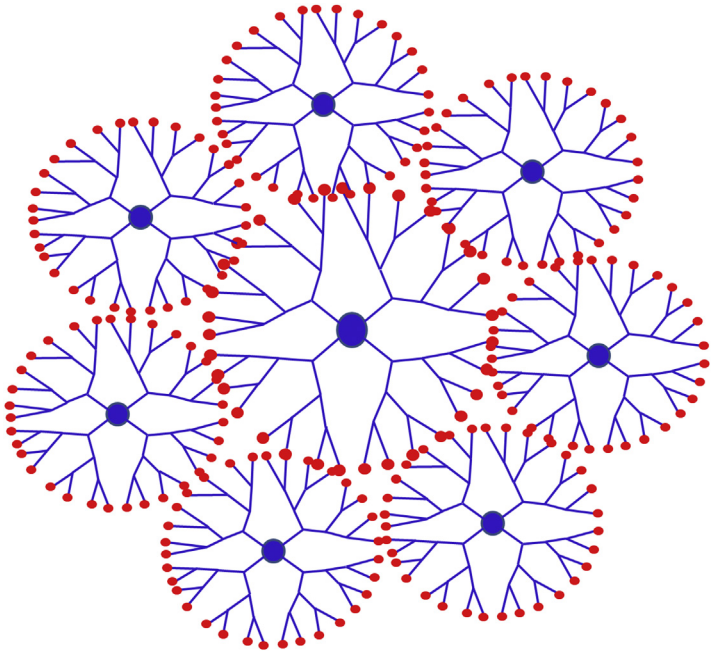

FIGURE 8.20 PAMAM tecto-dendrimer. *PAMAMOS*, Polyamidoamine organosilicon.

used to synthesize larger dendritic molecules without increasing the number of steps is synthesizing a core containing PAMAM dendrimer around which other PAMAM dendrimers are covalently attached as a shell. This new structure formed is known as core−shell tectodendrimers (Fig. 8.20). Generally, in comparison to the shell generation the number of cores is more. The certain supramolecular structure can also be synthesized with the help of dendrons. The basic difference between them and tectodendrimers is that they are formed by aggregation or association, while tectodendrimers contain covalent bonding (Betley et al., 2002). Tectodendrimers are used to synthesize a large dendritic molecule that mimics the protein structure without increasing the number of steps required for synthesis.

8.2.9 Poly(propylene imine) dendrimers

PPI dendrimer possesses hydrophobic core and high surface charge density (Liu et al., 2014). PPI dendrimers are generally synthesized by the divergent approach (Fig. 8.21). EDA is used as a core molecule on which −CN-terminated branches can be added by Michael addition reaction with the help of acetonitrile. This will produce half-generation dendrimer. Full-generation dendrimer can be produced by Raney nickel catalyst by using heterogenous hydration method. It will produce G1 dendrimers with a terminal amino group (Gajbhiye et al., 2013).

In comparisons to other dendrimers such as PAMAM or polyethyleneimine, the transfection capabilities of PPI are less. Hence, different approaches are used to improve the transfection capabilities. PPI dendrimers generally form stable noncovalent complexes

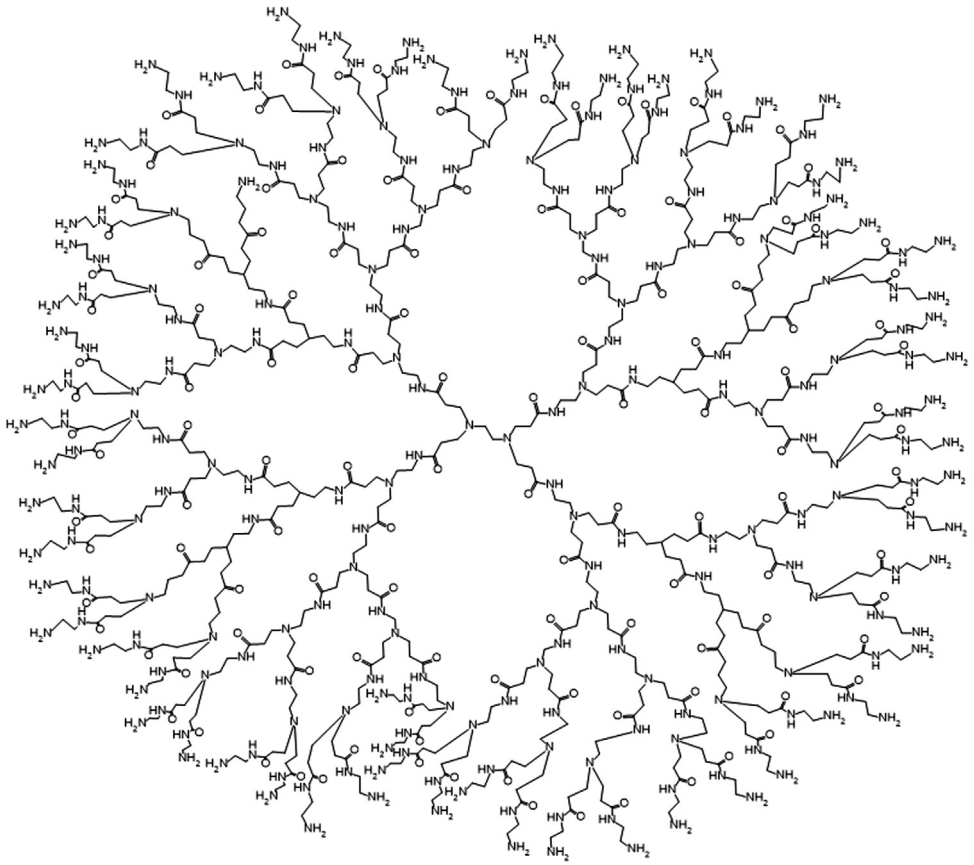

FIGURE 8.21 G4.0 PPI dendrimer. *PPI*, Poly(propylene imine).

with anionic drugs and nucleotides owing to their cationic terminal amino group. They are modified with sugar groups to remove their inherent toxicity, making them biocompatible, and improving their bioavailability (Klajnert et al., 2008). PPI due to the availability of many amino (NH_2) functional groups on the surface can serve as an important moiety for brain targeting (Patel et al., 2016; Dwivedi et al., 2016).

8.2.10 Triazine dendrimers

1,3,5-Triazine is an important moiety, which can be incorporated in the dendrimer structure because of some unique properties, namely, biological activities, high stability on functionalization, easy scalability with higher yields (Vembu et al., 2015). The different reactivity of various amine nucleophile with chlorotriazine allows developing diverse dendrimers (Lim and Simanek, 2012). Triazine-based dendrimers have also been found to show antibacterial activity with good chemical stability, less volatility, and nonirritant to the skin. Hence, due to these properties, they prove to be a good carrier for delivery of

antibacterial agents (Vembu et al., 2015). Melamine that contains 1,3,5-triazine skeleton is an important molecule studied for this class. Melamine-based dendrimers have been employed as a candidate for functionalization of carbon fibers. Further, they offer some advantages such as cost-effectiveness, easy purification after the end of the process as all the reagents can be easily washed off (Zhao et al., 2017).

8.2.11 Polylysine dendrimers

Dendrimers have been explored for their biodegradable nature. Polylysine dendrimers offer an advantage of biodegradability by enzymes present in the body as well as easy renal elimination of low molecular weight compounds. The rate at which biodegradation can take place depends upon the surface amino groups present and removal of capping groups. It offers a potential route for pH-dependent or enzymatic cleavage of the drug and carrier. They also serve as an important carrier for the delivery of anticancer drugs (Kaminskas et al., 2011; Tekade et al., 2009b).

8.3 Types of dendrimers

8.3.1 Drug-loaded dendrimer as nanovehicles

A dendrimer is a tree-like structure where the trunk of a tree can be assumed as its core, from which all the branches are originating, leading to other branching units (interior shells). These units further terminate as leaves, which can be related to surface functional moieties in case of dendrimers (Fig. 8.22). Increasing the number of branches around the core creates crowding, hence shielding the core from the outside environment. The interior shells and branches formed are responsible for holding the guest molecule inside the dendrimer by "click in" mechanism, thereby encapsulating the drug molecules by noncovalent (i.e., hydrophobic, physical entrapment, or ionic) interactions (Fig. 8.23). Further, the resultant weak interaction property can be helpful in the release of drugs at tumor sites, where a pH less than seven leads to protonation of amide groups, directing to its separation from the host molecule, hence deliver the drug at the tumor site (Tekade et al., 2009a).

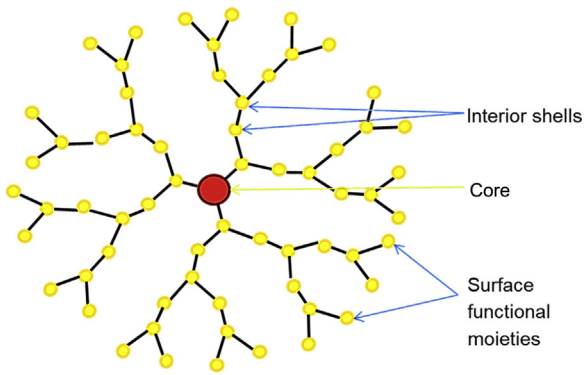

FIGURE 8.22 Architectural configuration of a simple dendrimer.

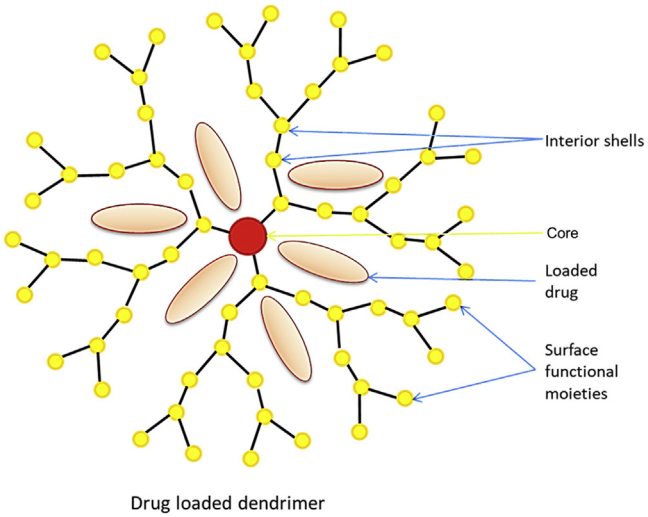

FIGURE 8.23 Architectural configuration of drug-loaded simple dendrimer.

Biocompatible monomers such as succinic acid and glycerol have been prominently used by Grinstaff et al to develop polyether-ester dendrimers known as poly(glycerol−succinic acid) (PGLSA) dendrimers. They used the phenomena of solvatochromism (i.e., Reichardt's dye) to study the internal environment of PGLSA dendrimers. However, in an analysis using the proton nuclear magnetic resonance (^{1}H NMR), the complex confirmed aliphatic protons from the dendrimers as well as aliphatic protons from the Reichardt's dye. But ^{1}H nuclear Overhauser effect spectroscopy (NOESY) spectra of the complex displayed a vast number of intermolecular NOESY cross-peaks implying close proximity of the dye with the dendrimers leading to the restricted motion of the encapsulated dye. This property can be potentially utilized in the formulation of many hydrophobic anticancer drugs where the drugs can be encapsulated inside the G4.0 PGLSA dendrimers and used as a drug-delivery device. For instance, 10-hydroxy camptothecin showed a significant reduction in viability of tumor cells in cytotoxicity assays of human breast cancer cells even when encapsulated inside G4.0 PGLSA dendrimers (Tekade et al., 2008a).

Altering the generation and surface charges of dendrimers can lead to various effective properties such as the following:

1. The increment in the generation of dendrimers leads to an increase in aqueous solubility of drugs such as paclitaxel by polyglycerol dendrimers (PGDs). Thus solubility in all the solutions of PGDs (even <10% concentration) was much elevated than PEG_{400}, which is a commonly employed cosolvent and hydrotropic agent. This can be demonstrated by ^{1}H NMR spectra indicating surrounded PGDs (Ooya et al., 2004). Therefore many dendritic polymer conjugates have been patented by Dow Chemical Company, which can be used for the delivery of antineoplastic drugs at tumor sites. These can be administered either orally, parenterally, or topically in sufficient amounts (Malik et al., 2003).
2. Increasing the generation of PAMAM dendrimers leads to an increase in permeability as well as cytotoxicity (Jevprasesphant et al., 2003).

Among cationic and anionic dendrimers, cationic dendrimers (G2.0, G3.0, G4.0) were found to be more toxic at the cellular level than that of anionic dendrimers (G2.5 and G3.5). This is because the apparent permeability coefficient (P_{app}) of cationic dendrimers at 37°C was greater than anionic dendrimers. But conjugating with lauryl chloride reduces the cytotoxicity of cationic dendrimers. The increment in P_{app} values can be achieved by increasing the number of attached lipid chains. Further, the value of P_{app} was observed higher in the presence of ethylenediaminetetraacetic acid, while it was found lower in the colchicine. However, it showed lower P_{app} at 4°C as compared to 37°C (Jevprasesphant et al., 2003).

8.3.2 Simple dendrimers in gene transfection

Gene therapy aims at correcting defects at a genetic level by transferring active genes into the target cells (Wiwattanapatapee et al., 2000). For this, transferring them directly through the bloodstream is not possible as nucleic acids (NA) are prone to be degraded by enzymes. Therefore a carrier is required for transfection of genes; earlier viruses were used as vectors, but due to their large implications (e.g., immunogenic proteinaceous capsid), their use has been restricted (Favre et al., 2001; Kay et al., 2001; Timme et al., 1998). In this regard, for being an ideal DNA vector, it should have following characteristics such as target specificity, high transfection efficiency, easily biodegradable, stability, less potential to be toxic or immunogenic, easy design, and synthesis. For the transfection of genes, dendrimers were the preferred choice, because this complex prevents NA from enzymatic degradation as well as it allows endosomal escape. The analogy of lipoplexes and polyplexes (lipoplex-complex of NA with liposomes; polyplex-complex of NA with linear polymers) was followed to develop dendriplexes (Domb et al., 2005). Dendriplexes are dendrimers with positively charged groups, which are able to bind with NA (Fig. 8.24). They have the ability to bind a large number of genetic materials due to their numerous ionizable end groups (Chen et al., 2000).

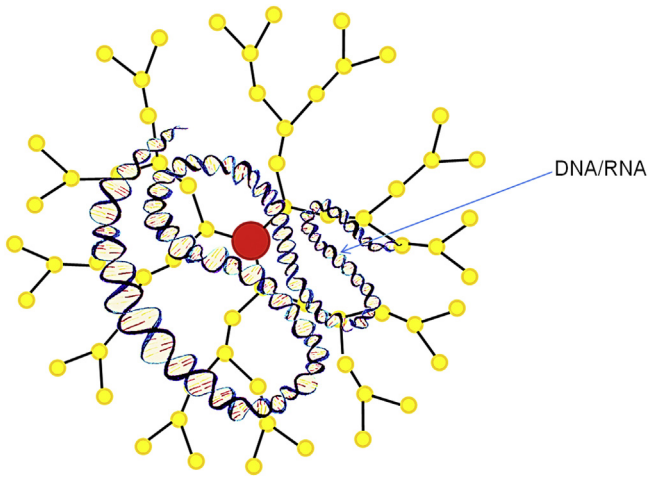

FIGURE 8.24 A structural depiction of dendriplex.

Various factors affect the binding ability such as the following:

1. Increasing generation of dendrimers doubles NA binding (Chen et al., 2000).
2. PAMAM dendrimers were found to be less toxic and more efficient for the genetic material transfer, than higher generation PPI dendrimers (Gebhart and Kabanov, 2001; Malik et al., 2000b).

Lower generations (i.e., G1.0−G2.0) with partially compacted PPI dendrimers efficiently transferred the genetic material with less toxicity as compared to higher generations (i.e., G3.0−G5.0) containing compact and condensed DNA (Zinselmeyer et al., 2002). Water-soluble phosphorous dendrimers can also be used for the efficient delivery of genetic material. Transfection efficiency is increased as we increase the generation from G1.0 to G3.0 (Loup et al., 1999). Further, single-stranded antisense oligodeoxynucleotides (ODNs) are delivered into cells for blocking mRNA. In this regard, agents such as PAMAM dendrimers can be used for the transfection of antisense ODNs along with reduced toxicity (Bielinska et al., 1996). In response to the dendriplex introduction the efficiency was increased 14-folds as compared to free ODN, and the expression of the gene was inhibited (Santhakumaran et al., 2004).

RNA interference can also be used as a method for inhibition of gene expression. It is based on the ability of the double-stranded RNA to undergo specific degradation of mRNA, which is having a complementary sequence to that of another RNA strand. As this double-stranded RNA enters inside the cell, endonucleases cleave them into short 19−21 bp stranded fragments (Zamore et al., 2000). From these studies, it can be concluded that dendrimers could be a promising vehicle for gene delivery. Several investigations have revealed that the increase in the dendrimer generation, as well as thermal treatment (which destroys few branches by solvolysis), results in an improvement in strand mobility, which also increases the transfection efficiency of PAMAM dendrimers. These activated molecules are called fractionated or degraded PAMAM dendrimers, commonly known as Superfect (Tang et al., 1996). They have higher efficiency for the transfer of genetic material than intact ones owing to the compactness and higher mobility (Praetorius et al., 2008).

A major issue associated with dendrimers can be resolved by following ways:

1. Increased generation of PAMAM dendrimers leads to cytotoxicity, which can be reduced by modifying the surface of dendrimers (Luo et al., 2002).
2. Incorporation of hydrophobic molecules to the surface of the dendrimer can influence the stability of complexes between NA and dendrimers surface with increased dendriplex and cells interaction.
3. On the other hand, PAMAM dendrimers conjugation with cyclodextrins can also improve its gene transfer efficiency and form complexes, which can protect them against deoxyribonuclease I degradation (Arima et al., 2001).

Amphiphilic lipid dendrimer hybrids were also developed with the help of cationic lipids and dendrimers having 10-fold efficiency of transferring plasmid DNA (Al-Jamal et al., 2005; Joester et al., 2003; Guillot-Nieckowski et al., 2007). Another upcoming trend involves internal quaternization of the groups on dendrimers. In this, they neutralized the surface of hydroxyl groups, so that their cytotoxicity can be reduced, whereas the cationic charges of dendrimers offer NA binding. These quaternized dendrimers along with

small interfering ribonucleic acid (siRNA) form globular dendrimers possessing higher transfection efficiency as they reached in the cytoplasm and nucleus unlike cationic PAMAM−NH_2 nanothreads. Eventually, cationic dendrimers had higher transfection efficiency over anionic and neutral dendrimers, since they interact with the anionic surface, which facilitates its penetration inside the cells (Muniswamy et al., 2019; Tekade et al., 2015a). Moreover, surface tailoring of dendrimers with specific moieties can improve the loading of siRNA and increase targeting potential by offering enhanced permeability, transfection, endosomal escape facilitation (Tambe et al., 2017).

8.3.3 PEGylated dendrimers in cancer therapy

PEGylation simply means the conjugation of PEG to the dendritic scaffold (Gajbhiye et al., 2009b). PEGylated dendrimers (Fig. 8.25) are more efficient than others in terms of drug loading, targeting, and solubilization (due to attached PEG chains). Moreover, they do not have hemolytic toxicity, short half-life, and their macrophageal uptake has decreased immunogenicity, antigenicity, and toxicity. Due to their various advantages, they can be used in anticancer drugs to target the tumor site. Thus PEGylating a dendrimer helps improving its kinetic stability due to surface crowding, thereby making it useful for extended delivery (Bhadra et al., 2002). A hybrid of drug-conjugated PEGylated dendrimers can be synthesized by incorporating hydrophobic drugs inside and attaching polyethylene oxide (PEO) moieties to the periphery of the dendrimers (Bhadra et al., 2002).

PGDs are more helpful in increasing solubility due to a large number of ethylene glycol units, while dendrimers generation can also affect the solubility of poorly water-soluble

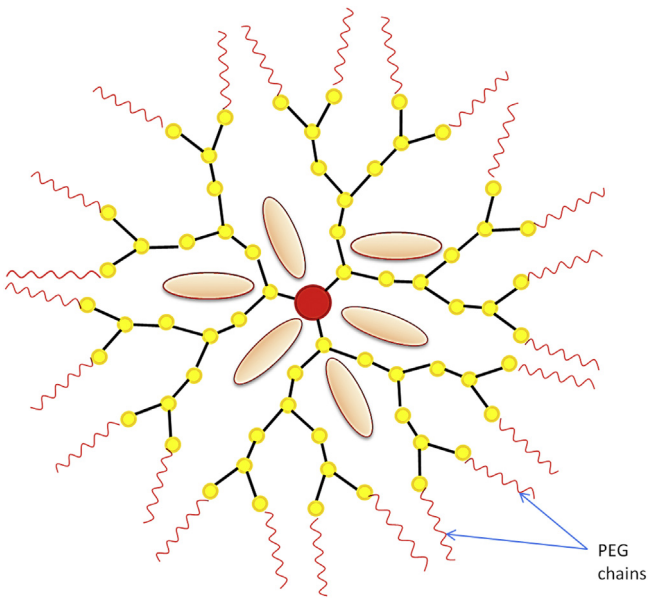

FIGURE 8.25 Architectural configuration of drug-loaded PEGylated dendrimers.

drugs (e.g., paclitaxel) due to the increased local density of ethylene glycol units (Ooya et al., 2003). This can be demonstrated by viewing ^{1}H NMR spectra of paclitaxel prior to and after mixing of PGDs in D_2O. It can be viewed that the PGDs surround the aromatic and methylene groups present on paclitaxel, thereby providing hydrotropic solubilization (Ooya et al., 2004).

By having simpler modification, many advantages can be obtained:

1. Solubilization of drug along with its sustained release action can also be achieved by combining the chain ends of G3.0 and G4.0 PAMAM dendrimers and PEG monomethyl ether via urethane bonds. Further, it was observed that the encapsulation of drug increases with an increase in the generation of dendrimers and chain length (Kojima et al., 2000).
2. PEGylated G2.0 and G3.0 PAMAM dendrimers can also be utilized as magnetic resonance imaging (MRI) contrast agents by incorporating gadolinium (Gd) ions, thereby providing an aqueous soluble monodispersed system. These contrast agents tailored with PEG were having prolonged circulation time and reduced accumulation (Kojima et al., 2011).
3. Their stability and blood residence time can be improved by attaching aspartic acid units along with arabinofuranosylcytosine (Ara-C) via its amine groups to form amides or carbamates (Choe et al., 2002).
4. The serum half-life of drugs such as DOX can be increased by linking via hydrazone to get high molecular weight three-arm PEO-dendrimer hybrid (Padilla De Jesús et al., 2002).
5. PEGylation also affects biodistribution and physiochemical properties of sixth-generation lysine dendrimers due to changes in particle size, ζ-potential, organ distribution, and blood retention (Okuda et al., 2006).
6. Conjugating a dendriplex with higher molecular weight PEG and higher generation dendrimers increases its transfection efficiency. Higher transfection efficiency and increased cellular uptake can be clearly visualized via confocal microscopy and flow cytometry of fluorescein-labeled DNA (Fig. 8.26).

In conclusion, PEGylation helps in achieving many properties as mentioned previously and also reduces hemolytic toxicity due to the decreased interaction of charged quaternary ammonium ions and RBCs by PEG chains.

8.3.4 Liposomal "locked in" dendrimer

It is the combination of liposomes and dendrimers, where dendrimers are locked inside the two layers of liposomes (Fig. 8.27). The size of PAMAM dendrimer exists in nanometer range which is equivalent to the thickness of the aqueous layer present in between two liposomal bilayers. Hence, dendrimers can be easily encapsulated in this space where its positive charges interact with the oppositely charged lipids (Purohit et al., 2001). The reason behind incorporating dendrimers can be justified as follows: In the case of liposomes, dilution or application can result in leaching out of hydrophobic or membrane permeable drugs. Therefore they have to be immobilized through "locked in" dendrimers. Also, higher drug loading and slower release can be achieved via liposomal "locked in" dendrimers

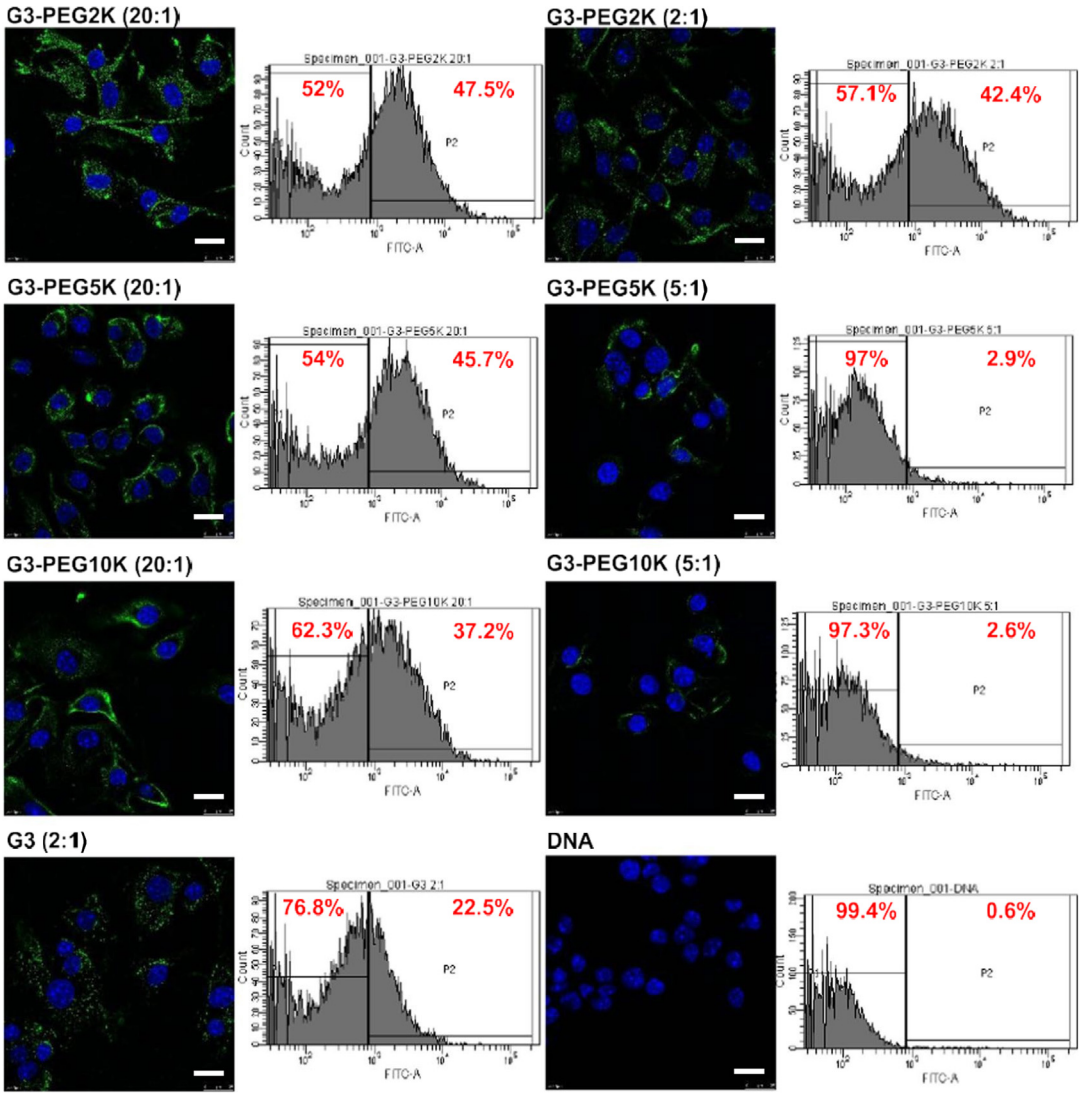

FIGURE 8.26 Confocal microscopy imaging along with flow cytometry quantification of the cellular uptake of fluorescein-labeled DNA (2.5 μg/well) complexed with G3-PEG2K (dendrimer:DNA weight ratios: 10:1 and 2:1), G3-PEG5K (dendrimer:DNA weight ratios: 20:1 and 2:1), G3-PEG10K (dendrimer:DNA weight ratios: 20:1 and 5:1), G3-DAB (dendrimer:DNA weight ratio: 2:1) or left uncomplexed, after incubation for 1 h with B16F10Luc cells [blue: nuclei stained with DAPI (excitation: 405 nm laser line, bandwidth: 415–491 nm), green: fluorescein-labeled DNA (excitation: 543 nm laser line, bandwidth: 550–620 nm)] (bar: 10 μm). Source: *Adapted with permission from Somani, S., Laskar, P., Altwaijry, N., Kewcharoenvong, P., Irving, C., Robb, G., et al., 2018. PEGylation of polypropylenimine dendrimers: effects on cytotoxicity, DNA condensation, gene delivery and expression in cancer cells. Sci. Rep. 8, 9410.*

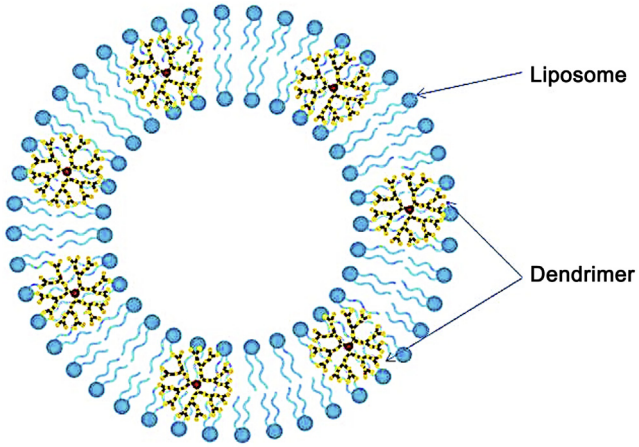

FIGURE 8.27 Diagrammatic representation of liposomal "locked in" dendrimers.

(LLDs). Encapsulation of drug inside LLD occurs due to dendrimer present between the layers, and as the generation of the dendrimer is increased, the amount of encapsulated drug also increases (Gardikis et al., 2010). For better in vitro release results, hexadecyl phosphocholine (ether lipid having antitumor activity), egg phosphatidylcholine, and stearyl amine in the molar ratio of 10:10:0.1 were utilized in forming liposomes attached to DOX–PAMAM dendrimer complex (Papagiannaros et al., 2005).

Therefore these "locked in" dendrimers can serve many advantages:

1. It increases the stability of liposomes.
2. It forms liposomal-controlled release systems.
3. It has decreasing side effects.
4. It has increasing therapeutic index.

8.3.5 Targeted dendritic scaffolds

Targeting implies delivering a drug to a specific site which is in need of treatment, thereby protecting the surrounding normal cells from being affected. Thus it leads to reduced dose and toxicity of the drug. These dendrimeric scaffolds can be used for drug targeting. It has many more advantages that can be utilized for effective drug delivery like good aqueous solubility (Svenson and Chauhan, 2008), low polydispersity index (PDI), devoid of immunogenicity (Yang et al., 2008), as well as a large number of amine groups, are present that can be modified. They can also be used to combat multidrug resistance. Due to their small size as that of serum proteins, they can cross vascular pores and reach tumor cells. These can be conjugated with FA, glycodendrimers, monoclonal antibody, or RGD to achieve tumor targeting.

8.3.5.1 Deoxyribonucleic acid–assembled dendrimer–folate conjugates

For targeting tumor cells, multifunctional groups such as FA are required on the surface of a dendrimer for which receptor is overexpressed in tumor cells. Therefore Choi and Baker produced dendrimers conjugated with different biologically functional moieties such as

fluorescein isothiocyanate (FITC) and FA and then linked them with complementary DNA (cDNA)−ODNs to get cluster molecules for targeting cancerous cells, which have an affinity for FA (Choi and Baker, 2005). Thus ODNs were hybridized with conjugates of FITC and FA. Their specificity for a subline of the ubiquitous KERATIN-forming tumor cell line HeLa (KB) cells was confirmed by in vitro studies, while the internalization of this crystal can be demonstrated by confocal microscopy. Thus this nanocluster is a breakthrough for the delivery of genetic materials and theragnostic purposes (Bayele et al., 2006).

8.3.5.2 Multimodality dendrimer-based diagnostic agents

During the formulation of imaging or diagnostic agents, it is always necessary to keep in mind the target specificity of that agent. Various macromolecular contrast agents with active targeting potential can be utilized to attain selectivity and combating the nonselective accumulation of the drug in tumor owing to enhanced permeability effect via passive targeting (Jain, 1987). Due to elevated production costs and potential immunogenicity, the formulations such as Gd-diethylenetriaminepentaacetic acid (Zhou and Lu, 2013) and antibody-conjugated paramagnetic liposomes were discontinued owing to the failure to achieve the desired results (Madaan et al., 2014). In this regard, to combat the concerning issues, for example, size of the diagnostic agent, a wide range of surface-tailored dendrimers came into the role (Soni et al., 2017). Their imaging effect for extended time times can be attained by exploiting their larger size (Patri et al., 2002; Lo et al., 2013).

To develop MRI contrast agents possessing high activity, Wiener et al. developed contrast agents to target overexpressed folate receptors. Further, the effect of folate-attached PAMAM dendrimers with TU-DTPA (thiourea diethylenetriaminepentaacetic acid) was determined via forming folate-PAMAM−TU-DTPA. Fluorescence studies can be done by reaction with FITC and carboxytetramethyl rhodamine succinimidyl ester (Wiener et al., 1997). Moreover, to attain enhanced antitumor activity due to EPR effect, polymer−drug conjugates of paclitaxel using poly(L-glutamic acid) (PGA) were developed by Li et al. (Li, 2002; Li et al., 1998). Further, this conjugate was attached to dendrimers, and their activity toward active targeting as well as EPR effect was monitored. In presence of lysosomal enzyme cathepsin B, this conjugate containing PGA is degraded leading to slower release than linear PGA. Diagnostic agents such as indocyanine green, which is a near-infrared dye, can also be conjugated to these scaffolds (Tansey et al., 2004). The solubility issues related to FITC and folate-conjugated dendrimers can be resolved by using fluorescein as a core and targeting with folate on the surface (Kesharwani et al., 2014a). These tectodendrimers provides superior and water-soluble dendritic polymers (Uppuluri et al., 2000).

8.3.5.3 Glycodendrimers in cancer targeting

Glycodendrimers (i.e., carbohydrate containing dendrimers) belong to the family of neoglycoconjugates. Carbohydrates—oligo and polysaccharides—present on the outer surface of cells are responsible for the cell's interaction with the surrounding (Fig. 8.28). These interactions can be seen during invasion or colonization of pathogens or during metastasis of aberrant glycosylated cancerous cell. Extracellular cells, such as natural killer CD8 + and CD4 + lymphocytes, recognize these glycosylated cancer cells with the help of lectin protein receptors (Turnbull and Stoddart, 2002). For enhanced affinity toward the binding,

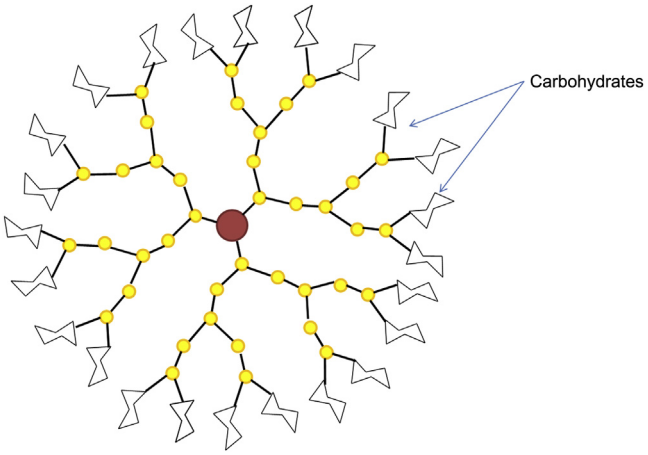

FIGURE 8.28 Diagrammatic representation of glycodendrimers.

these carbohydrates should be clustered. Therefore to promote clustering and reduce immunogenicity, glycodendrimers are constructed.

Glycosylating a dendrimer decreases its innate cytotoxicity and increases their specificity (Gorzkiewicz et al., 2018). T-Ag glycol PAMAM dendrimers were synthesized using amide bonds, revealing potentially strong protein binding leading to enhanced cluster effect, which can be used against cancerous cell metastasis (Jain and Tekade, 2013; Baek and Roy, 2002). The antitumor and anti-HIV activities can also be increased by sulfation of these oligosaccharides. A single dose of glycocluster can replace even several injections of neoglycolipid-coated liposomes (Pospisil et al., 2000; Pospisil et al., 2001). On comparing multivalent and monovalent antigens, the inhibitory effect of multivalent conjugates was quite larger than monovalent antigens (Roy et al., 2001).

Octavalent PAMAM dendrimer containing *N*-acetylglucosamine residues (PAMAM-GlcNAc8) on its surface can be used to stimulate an immune response against tumor cells. The presence of *N*-acetylglucosamine increases their affinity for NKR-P1 lymphocyte receptor (Vannucci et al., 2003). Further, water-soluble dendrimers can also be formed using G3.5 PAMAM dendrimers conjugated with D(+)-glucosamine to achieve immunomodulatory, while conjugating with D(+)-glucosamine 6-sulfate impart antiangiogenic properties (Shaunak et al., 2004). In a recent study, fludarabine triphosphate was incorporated inside the maltosylated open-shell PPI dendrimer. The outcomes revealed the elevated cytotoxic activity of dendrimers toward cancer cells and also diminished the drug resistance (Gorzkiewicz et al., 2018).

8.3.5.4 Arginine–glycine–aspartic acid–coupled dendrimers in antiangiogenic therapy

RGD is an integrin ligand, a tripeptide consisting of arginine, glycine, and aspartate. Its peptide sequence is present in many extracellular matrix proteins, for example, laminin, vitronectin, fibronectin, and collagen (Yang and Kao, 2007). Ligation of $\alpha_v\beta_3$ integrin (an extracellular matrix protein) is responsible for angiogenesis in tumor cells, where $\alpha_v\beta_3$ integrins are specific markers present on the surface of endothelial cells during angiogenesis in cancer cells (Desgrosellier and Cheresh, 2010).

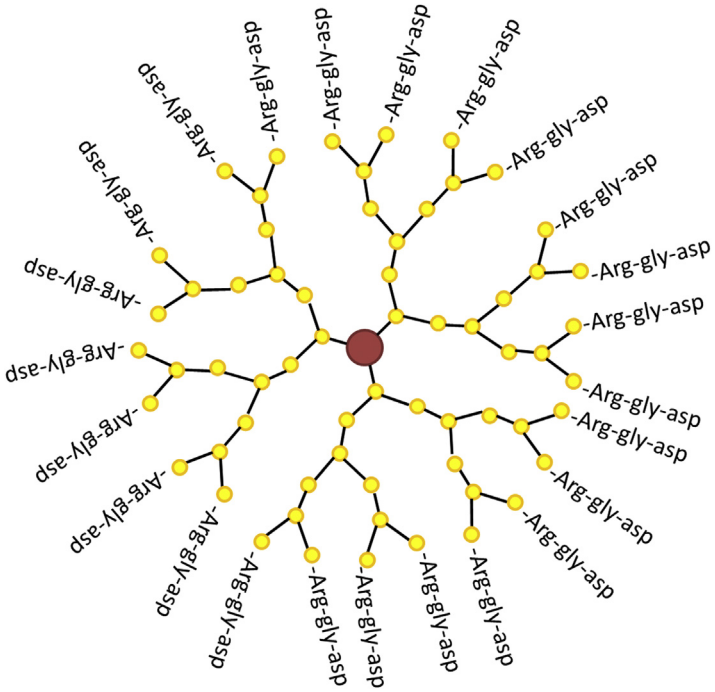

FIGURE 8.29 Schematic diagram of RGD-coupled dendrimers. *RGD*, Arginine–glycine–aspartic acid.

Inhibiting these $\alpha_v\beta_3$ integrins can lead to blocking of tumor-induced angiogenesis, or RGD peptides can mimic matrix proteins–producing antagonistic action. In the case of tumor cells, antiangiogenesis averts the neovascularization, thereby inhibiting their growth and providing anticancer activity. In this regard, dendrimers coupled with RGD peptide are synthesized (Fig. 8.29). Further, the amine groups or carboxylate moieties present on the surface of dendrimers can be covalently linked with RGD peptides. However, the coexistence of this peptide sequence with cytotoxic agents leads to tumor endothelial cell-selective targeting to kill the new vessels. Thus the uptake of this conjugate by cells is receptor-mediated, which suggests that the addition of any excess peptide could inhibit the uptake (Shukla et al., 2005). Thus the RGD-coupled dendrimers can be successfully employed as imaging and antitumor agents via antiangiogenic therapy. A recent study indicates their efficient delivery as a gene carrier possessing high specificity and reduced toxicity toward PC3 tumor xenografts inside the mouse models (Kim et al., 2017). In the future, RGD dendrimers can be used as a potential candidate for treating cancer and other genetic disorder.

8.3.5.5 Antibody/ligand-guided dendrimers

Antibodies are also known as immunoglobulins, which are proteins produced inside a host cell in response to a foreign molecule. They have the capability of recognizing other molecules efficiently with high specificity, which is the prerequisite of any detection

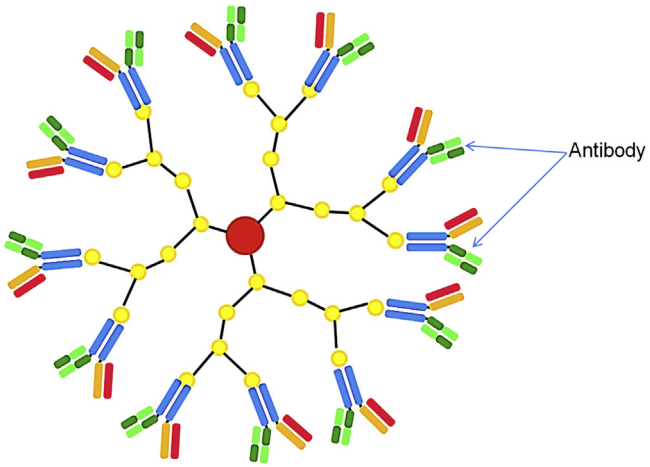

FIGURE 8.30 Schematic diagram of antibody-conjugated dendrimer.

method. Therefore they are being used in recent times as biosensors, catalysts, diagnostics, and gears of nanotechnology (Yamaguchi and Harada, 2003). They can be utilized against tumor cells since tumor cells express specific surface antigens, which are easily identified by antibody and make them vulnerable to immune cells. Or else, antibodies can destroy blood vessels that are a source of growth factors for tumor cells. But their targeting with drugs can lead to the loss of their biological activity and solubility.

To resolve these issues, dendrimeric-antibody [J591 anti-PSMA (prostate-specific membrane antigen)] conjugates (Fig. 8.30) containing fluorophores were employed (Patri et al., 2004), and 90% activity of an unmodified antibody was retained by forming PAMAM immunoconjugates (Tekade et al., 2009). Thus these antibodies containing dendrimer can be utilized against T-antigen in carcinomas, CD14, and PSMA with the help of 60bca and J591 antibodies (Thomas et al., 2004b). In a current approach for enhancing the cytotoxicity of gemcitabine, dendrimers conjugated with Flt-1 antibody were employed for antitumor activity against pancreatic tumor (Öztürk et al., 2017). Therefore antibody-guided dendrimers can be used as potential targeting therapeutic agents for delivery of anticancer drugs.

8.3.5.6 Dendrimers as multiprodrugs

In addition to the abovementioned types, dendrimers can also be modulated as multiprodrugs. Structural dendrimers can be formed by attaching various drugs as tail units and an enzyme substrate to the dendrimer core. A single enzymatic cleavage at the core can trigger the release of tail units from the core. DOX and camptothecin have been used as tail units to synthesize dendritic prodrugs and catalytic antibody 38C2 as a trigger for cleavage (Shamis et al., 2004). These dendritic assemblies can thus be used for a combination of personalized therapy as well as for theragnostics (Fig. 8.31).

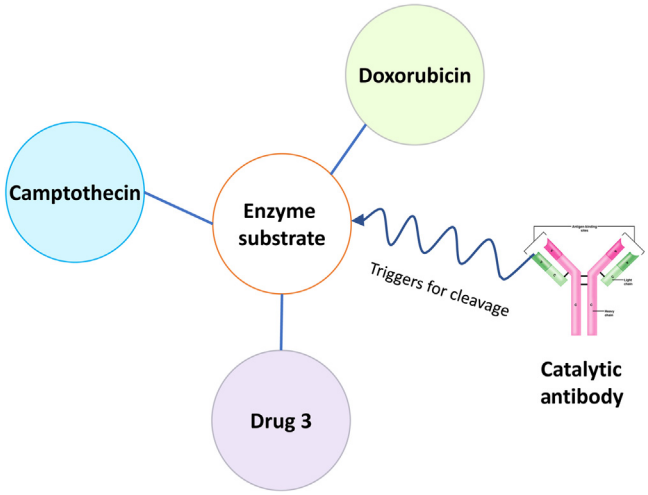

FIGURE 8.31 Schematic diagram of multiprodrugs dendrimer.

8.4 Applications of dendrimers

Dendrimers possess a structure that has the capability to be modified according to various applications. The multifunctionality of its surface and the internal cavity along with the core make it widely applicable in various fields such as biomedical, diagnostics, cancer therapy, and tissue engineering (Gorain et al., 2017). They also impart utility in drug delivery (Mody et al., 2014), gene delivery, oligonucleotide labeling, photodynamic therapy, vaccine delivery, boron neutron capture therapy (BNCT), and targeted drug delivery. Further, they mimic the nature of proteins; hence they can be modified into drug molecules. Thus the unique structure and features of dendrimers make them a potential candidate for using the following purposes:

8.4.1 Drug delivery

Dendrimers are proved to be an efficient drug-delivery system as they have a kind of structure that can be controlled, and their functional groups that are placed terminally are highly reactive in comparison to the other types of polymers. The PAMAM dendrimers are being explored for effective delivery of drugs through the oral route. They consist of branched structure, which is soluble in water. However, in solution, they depict a sphere-like compact shape (Hu et al., 2016). Recently, it has been reported that these dendrimers are capable of passing through paracellular as well as transcellular routes and can easily open tight junctions. Dendrimers are mostly being investigated as carriers for nonsteroidal antiinflammatory drugs. These lyophilic drugs are being solubilized by PAMAM or PPI type of dendrimers as they possess amino groups at the terminal portion. Thus their structure is able to encapsulate the drug (Markowicz-Piasecka and Mikiciuk-Olasik, 2016).

Biodegradable dendrimers are the most appropriate ones for developing novel drug-delivery systems for effective targeting. The drugs have been incorporated into

dendrimers via different ways such as by chemical conjugation, and some are encapsulated physically. Due to their biodegradable nature, they exhibit more importance and utilization in drug delivery (Huang and Wu, 2018). They offer the following advantages as a carrier in drug delivery:

1. The property to increase the residence time of drug by preventing their excretion through the kidneys.
2. Enhance the solubility of hydrophobic drugs and avoid their degradation in the internal fluid.
3. The ability to provide a targeting approach in the removal of the tumor.
4. Increasing the permeation of drugs and offer efficient targeting in the treatment of the tumor.
5. Ability to adjust the pharmacodynamics of drug by entrapping or conjugating the drug.
6. The ability to design controlled drug release systems.
7. More essential in minimizing side effects and decreasing the intensity of pain, hence establishing a painless platform in the form of drug carriers (Huang and Wu, 2018).

8.4.1.1 Drug–dendrimer binding mechanisms

The drugs bind with the externally placed groups of dendrimeric structure and interact with it. The incorporation of drug into dendrimer depends on the structure of dendrimer and the peripheral functional groups (Fig. 8.32). Hence, the generation of dendrimers governs drug–dendrimer interaction. The different ways through which dendrimers interact with drugs are as follows:

1. *Electrostatic binding*: The surface of dendrimers contains functional groups that possess certain charge, which attract drug molecules having opposite charge and thus forms an electrostatic bond. This type of binding also increases the solubility of the drug molecule. One example of such interaction is the binding of ibuprofen drug with

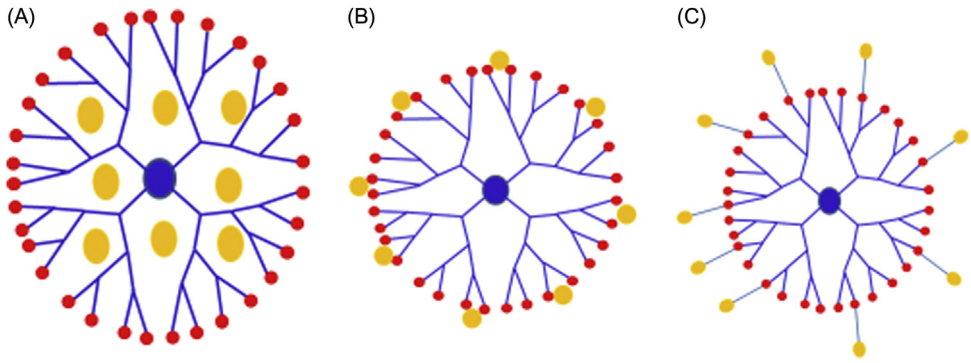

FIGURE 8.32 Drug–dendrimer binding mechanisms: (A) entrapment of drug through physical interaction, (B) drug adsorption on dendrimer surface, and (C) conjugation of the drug with dendrimer.

PAMAM dendrimer; it has been analyzed that G4−PAMAM dendrimers can incorporate 40 molecules of ibuprofen at pH ~ 10.2 (Sherje et al., 2018).

2. *Drug encapsulation by dendrimers*: Dendrimers are having unique structural features such as an open structure, which imparts them with the capacity to encapsulate various drugs either through the entrapment of drugs physically or nonbinding structural properties that promote interaction (Sherje et al., 2018).
3. *Dendrimer conjugated with the drug*: The type of conjugation that exists between the drug and dendrimer can be of either covalent or noncovalent. Certain agents, such as *p*-aminobenzoic acid and PEG, are being utilized for creating a covalent bond between the dendrimeric functional group at the terminal and the drug molecule. This type of binding makes the drug more stable and renders it suitable for controlled release. For instance, epirubicin was formulated as a prodrug by conjugating it with PEG dendrimers containing aminoadipic acid. This formulation reduced the degradation of the drug by making it more stable and improved its therapeutic efficacy by improving its residence time in the blood (Sherje et al., 2018).

Certain examples of dendrimers being used as drug carriers are as follows (Markowicz-Piasecka and Mikiciuk-Olasik, 2016):

1. PAMAM—flurbiprofen (i.v.);
2. PAMAM—ketoprofen (oral);
3. PAMAM—ibuprofen (transdermal); and
4. dendrimers consisting of PEG-citric acid copolymers incorporate the drug nimesulide for in vitro studies.

8.4.2 Oligonucleotide labels

ODNs are short sequences of DNA, which are used for the diagnosis of certain diseases and infections. The hybridization occurs between single-stranded DNA and cDNA to detect the change in a sequence, which leads to a certain disease or pathogenic condition. For this purpose, DNA sensors are being developed electrochemically. Dendrimers such as PAMAM offer great potential to be used as substrates for forming nanocomposites. The 3D structure and its flexibility render them useful for fabricating metals to construct sensors. Silver encapsulated dendrimers are being formed to develop oligonucleotide as labels for detecting hybridization of DNA (Jin et al., 2018).

8.4.3 Photodynamic therapy

It is a therapy that is employed in the treatment of cancer and to target specific sites of disease. It includes a photosensitizing agent in combination with drugs, and when it is irradiated with some source of light then it generates ROS that damage the surrounding cells. These photosensitizers have certain limitations such as less selective in nature, and they make the skin more sensitive to light. Therefore to combat such limitation, dendrimers are being widely worked upon to modulate and develop an optimum photodynamic therapy (Pandey et al., 2019). The photosensitizing agents can be incorporated into

dendrimers in several ways. They may bind with the terminally placed functional group, form a covalent bond with the core structure, or conjugate with the interior portion. Mostly PAMAM and PPI dendrimers are being used for encapsulating photosensitizers. Recently, phosphorous dendrimers are also developed and evaluated by some researchers to form photosensitive dendrimer conjugates (Militello et al., 2018).

Kojima et al. had synthesized PEG−PAMAM and PEG−PPI dendrimers to prepare nanocapsules for encapsulation of photosensitive agents such as Rose Bengal and protoporphyrin that resulted in the stabilization of these agents. Some research has also been conducted that involved modification of the acrylate group at the terminal of dendrimers to the allyl group that leads to the formation of thiol-ene structure (Kojima et al., 2007). This type of modified dendrimers requires less UV light due to the large availability of allyl groups to react with thiyl groups. Hence, skin is exposed to less light and it improves the curable effect. Thus improving the effectiveness of photodynamic therapy (Sharma et al., 2017).

8.4.4 Gene delivery

Dendrimers can serve as an important vector for gene delivery. Their flexible structure, terminal groups, and immunogenic property make them capable of conducting gene delivery. Mostly cationic dendrimers are being used as nonviral vectors for transfection of genes into the host cell. They prevent DNA and RNA from being degraded by enzymes as they have the ability to condense them. NA conjugate with dendrimers through multiple electrostatic bonds and then they are taken up into the internal structure through different endocytic mechanisms. PAMAM and PPI are the types of dendrimers that possess amine groups at terminal positions. As these tertiary groups have high density, they can be easily protonated in the endosomes at pH of 5.0−7.4. Therefore such dendrimer−NA conjugate (i.e., dendriplexes) are prone to experience "proton sponge" effect that finally leads to endosomal escape (Hu et al., 2016).

Research has been employed in the synthesis of dendrimers applicable in gene delivery, for example, triethanolamine (TEA)-cored PAMAM dendrimers glycoplexes, cyclodextrin PAMAM, G2 PAMAM, G9 PAMAM, dioleoyl phosphatidylethanolamine−PAMAM (Kesharwani et al., 2017). The G2 PAMAM dendrimers are highly efficient in transfecting as well as they are less cytotoxic. TEA-cored PAMAM is highly flexible and increase the binding of the dendrimeric structure with DNA. In addition, they create certain pores or spaces within the structure in order to increase the reactivity of terminal groups with water, which protonates them and accelerate their endosomal escape (Fig. 8.33) (Hu et al., 2016). Cyclodextrin PAMAM as compared to the nonfunctionalized PAMAM exhibited 100 times more expression of the luciferase gene (Kesharwani et al., 2017).

8.4.5 Boron neutron capture therapy

This therapy is used to treat many gliomas. It works on the mechanism that employs the irradiation of ^{10}B with neutrons having less energy to generate α particles and ^{7}Li nuclei, which have high energy. This type of interaction is called lethal $^{10}B(n,\alpha)^{7}Li$ capture reaction. These particles produced through BNCT traverse a very short path in tissues

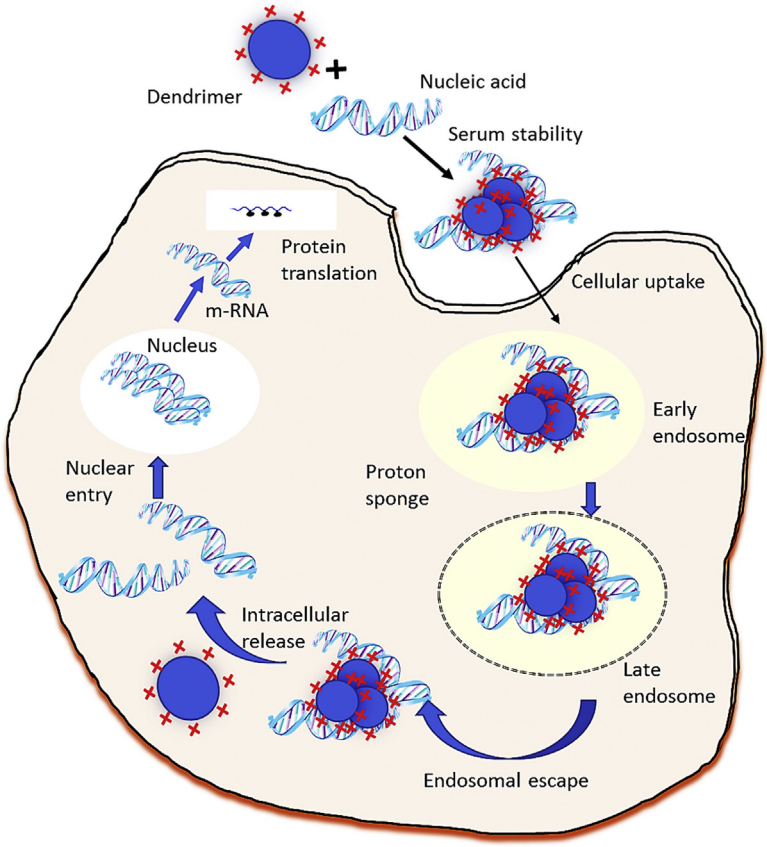

FIGURE 8.33 Transfection pathway of cationic dendrimers.

(b^{10} mm), and hence, they are less toxic and constricted to the internal cells. PAMAM is being investigated to deliver BNCT agents into the tumoral region. Conjugates of anti−epidermal growth factor (EGF)-antibody with boronated G5-PAMAM have been utilized for targeting some gliomas in humans (Fig. 8.34) (Sharma et al., 2017).

8.4.6 Small interfering ribonucleic acid delivery

siRNA is the field of research, which encompasses better targeting potential at the level of genes. But it has a major drawback, that is, owing to the negative charge, the diffusion of siRNA through the skin via passive transport is not feasible. Its molecular weight is high and has a hydrophilic nature. Moreover, it is prone to degradation by enzymes in plasma, which accelerate its clearance and thus reduce its uptake (Pandi et al., 2018). In order to combat the above problem, PAMAM dendrimers are found useful owing to the positive charge, which is due to the presence of amine groups at the surface. Further, their molecular weight distribution is also observed in a narrow range. Hence, the negatively charged NA can interact with the dendrimer at surface and form complexes. This type of complex

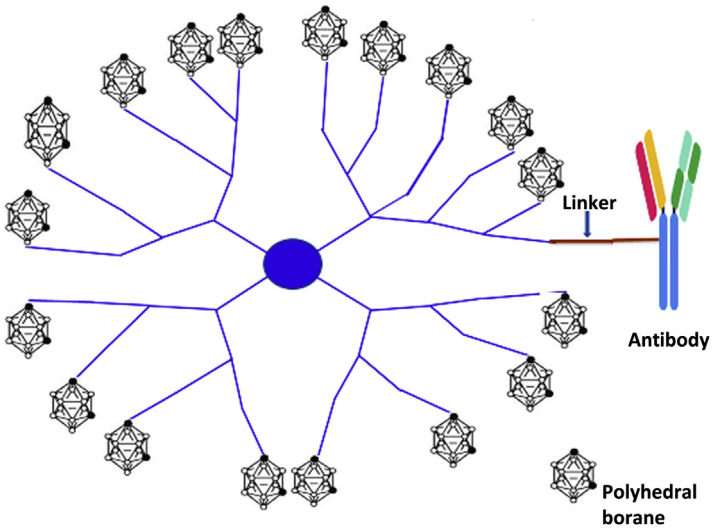

FIGURE 8.34 Monoclonal antibody-conjugated boron dendritic carrier for BNCT. *BNCT*, Boron neutron capture therapy.

formed in between dendrimer and a biomolecule is referred to as dendriplex. These dendriplexes were found to have more efficacy as compared to lipoplexes. Dendrimers act as nonviral vectors or carriers for siRNA delivery. Examples of dendrimers, which are being worked upon for this purpose, are poly(L-lysine), PAMAM, PPI, carbosilane dendrimers, polyglycerol-based dendrimers, triazine dendrimers, etc. (Pandi et al., 2018).

8.4.6.1 Intracellular delivery of survivin small interfering ribonucleic acid

Dendrimer structure can be combined with single-walled carbon nanotubes (SWCNTs) to deliver siRNA and conduct its imaging. Therefore dendrimers serve as an important aid in gene delivery, tumor targeting, and other therapeutic purposes. It is being reported that G4.0 dendrimer was modified and constructed into SWCNTs for mediating delivery as well as imaging of siRNA. The morphological characterization of SWCNTs and dendrimer-coated SWCNTs was analyzed through high-resolution transmission electron microscopy at 80 kV (Wang et al., 2013).

The carbon nanotubes whether single or multiwalled have certain issues such as cytotoxicity and biocompatibility. In order to reduce their toxicity and improve their biocompatibility, SWCNTs can be treated with certain functionalizing agents. This would further modify the surface of SWCNTs and enhance their surface morphological characteristics that could be utilized for various therapeutic approaches. They regulate the exposed cells that are mostly controlled according to the size and surface area of nanotubes. Therefore the G4.0 dendrimer-coated single-walled nanotubes were found to exhibit low cytotoxicity. They were electrostatically bound to the survivin siRNA vector for mediating the efficient entry of the resulting complex of SWCNT and survivin siRNA into MGC 803 cells. Then the survivin siRNA vector releases from the dendrimer-coated SWCNT−survivin siRNA

complex and further inhibits the growing capacity of tumor cells. Thus such dendrimer-based SWCNTs can be utilized for conducting the delivery of siRNA and its imaging for developing intracellular treatment strategies for cancer (Wang et al., 2013).

8.4.7 Oligonucleotide delivery

Studies are being conducted recently on dendrimers as agents for antisense oligonucleotide delivery. The 3D structure of dendrimers offers them a definite framework essential for delivering ODNs. Cationic dendrimer such as PAMAM has the affinity to bind with anionic ODNs. Thus they can be used for ODNs delivery. Anionic dendrimers are also being explored for conducting this type of delivery. A study was carried out to determine the efficacy of anionic dendrimer, for instance, the pentaerythritol was delivered using ODN into the cancer cells and specifically targeted the EGF receptor. It was observed that these anionic dendrimers increased the stability and uptake of ODNs into the cancerous cells (Hussain et al., 2004).

8.4.8 Targeted delivery

Dendrimers have shown its potential in targeting via both mechanisms, that is, active and passive targeting. Active targeting refers to the use of conjugated ligand-mediated systems that particularly bind to the target site and do not produce any off-target effect. Passive targeting is mediated via EPR effect, that is, enhanced permeation and retention effect. This phenomenon depends on the size of dendrimer employed for drug delivery. Hence, this mechanism involves the leakage of vascular blood vessels in tumor cells and facilitates effective targeting (Fig. 8.35) (Sharma et al., 2017).

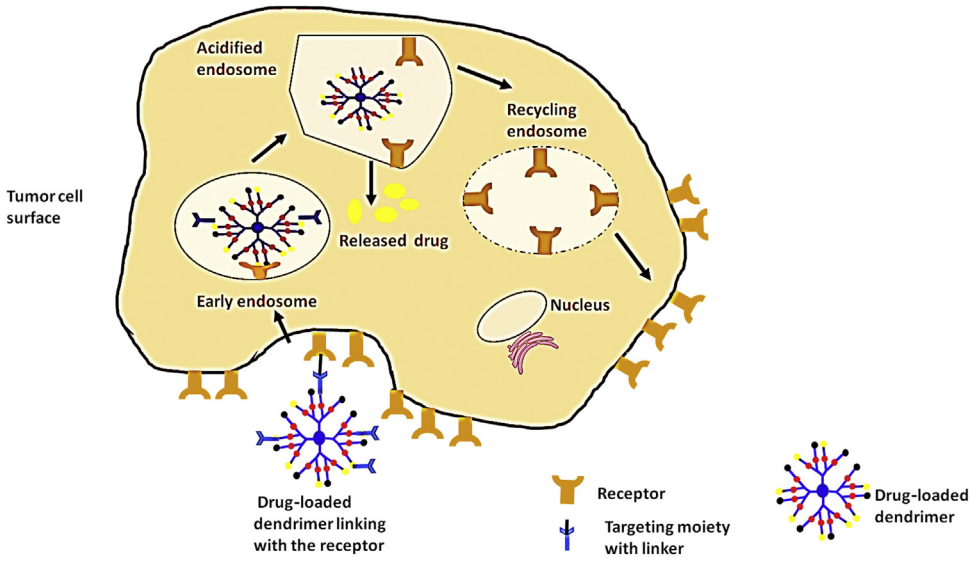

FIGURE 8.35 Mechanism of targeted delivery through dendrimers.

8.4.8.1 Folate-targeted

The targeting potential of dendrimers has been identified for binding to the overexpressed receptors for FA in tumor cells (Kesharwani et al., 2015b). G5-PAMAM dendrimers have been reported as a binding agent that has a high affinity for a large number of FA moieties. This approach of dendrimers is being utilized for developing receptor-mediated delivery of anticancer drugs, for example, methotrexate (folate antagonist). FA-conjugated G5-PAMAM incorporating methotrexate is reported to have more specificity toward receptors in KB cells. Recently, sensors of PAMAM dendrimers are also being developed to determine the effectiveness of anticancer drugs by targeting tumor cells (Wolinsky and Grinstaff, 2008).

8.4.8.2 Dextran targeted

Dextran has also been investigated as a drug-delivery carrier by conjugating it with dendrimer. It is specially designed to target the mass of the tumor cell. In a recent study a PPI–dextran conjugate of DOX was prepared. This was found to be very effective in delivering the drug to the tumor site without modifying the chemical properties of the drug. The molecular weight of dextran is high enough to impart its longer residence time in blood. Further, it reduces its clearance and raises the half-life of the drug. DOX is the drug used for the treatment of breast cancer, but it has certain limitations such as toxicity, side effects, and narrow therapeutic window. These shortcomings can be overcome by conjugating it with dextran–PPI, which would enhance the localization of the drug within the tumor cell by passively crossing the porous membrane of tumor cell (Agarwal et al., 2009).

8.4.8.3 Carrier for bone targeting

Yamashita et al prepared a dendrimer conjugate, that is, PEG-G3-PAMAM, which had a bone targeting potential to treat various bone diseases. The backbones of PAMAM dendrimers were conjugated with four types of carboxylic acids, that is, succinic acid, aspartic acid, glutamic acid, and aconitic acid. PEG was covalently linked with the (COOH)-group of carboxylic acid and amine group of PAMAM dendrimer. A distribution study was conducted in the internal compartments of bone, which demonstrated that fluorescein isocyanate–labeled PEG conjugate of PAMAM–aspartic acid had a wide distribution on inactive surfaces of bones, which are prone to undergo erosion in bone ailments such as osteoporosis and rheumatoid arthritis. Therefore these types of modified PAMAM dendrimers can be considered novel carriers for bone targeting (Yamashita et al., 2017).

8.4.9 Vaccine delivery

Dendrimers possess some desired attributes to conduct efficient vaccine delivery. They play an important role as a carrier for antigens, which leads to the development of multimer conjugates of antigen. These conjugates do not produce undesired host responses and impart the essential properties to be used for human beings. Further, they also do not elicit any type of inflammatory or immunogenic response when in use. PAMAM type of dendrimers as such do not produce any antigenic response, but when they are modified through conjugation with the protein, they develop an antigenic response, which is PAMAM dependent (Parajapati et al., 2016). A dendron comprising of lysine units was reported by

Tam. Every single unit of lysine consists of two types of amino acids, that is, R and ε, which act as determinants for branching. Tam thus gave the term "Major antigenic peptide" that is known as MAP, which constitutes two and three dendron layer equally distributed between both the amino groups. This type of structure is not restricted to peptides, but even small size moieties can bind to the amino groups placed terminally at the MAP core (Parajapati et al., 2016; Tam, 1988).

8.4.10 Tissue engineering

The dendrimeric structure favors the suitability of dendrimers to be applied as an essential agent in various biomedical purposes. One such application is that of its use in tissue engineering as they can incorporate both hydrophilic as well as lipophilic drugs and conduct site-specific delivery. They can be constructed with the desired nontoxic attributes in the 3D scaffold surface that serves as a platform for the growth of cells in the extracellular matrix containing the essential biomolecules, growth factors, and hormones (Gorain et al., 2017).

8.4.11 Imaging

Various diagnostic techniques, such as MRI and computed tomography (CT) scan, are being employed to detect and diagnose different organs affected with the specific diseases. This type of diagnosis requires a certain contrasting or diagnostic agent. Dendrimers due to their modulating structure can be conjugated with such agents in order to improve their diagnostic efficiency. PAMAM dendrimers labeled with Gd have been in research for quite a long time as carriers to enhance clearance, targeting ability, and contrasting features of Gd. It has been used to observe the structure of the tumor and determine the lymphatic associations present at the target site. Further, Gd is also being complexed with PPI and tested for its use as a contrasting agent in MRI. These dendrimers have a high molecular weight; therefore they have the capability to retain themselves in the blood for a longer period of time, hence provide more effective visualization and detection of the tumor (Wolinsky and Grinstaff, 2008).

Fluorescent tubes are mostly being used for tumor diagnosis as they are biocompatible, but they have a limitation, that is, the light is not able to penetrate the tissue effectively. Therefore to resolve such issue, Thomas et al developed a conjugate of FA, G5-PAMAM, and a fluorescent probe, that is, 6-carboxytetramethylrhodamine (6TAMRA). It was designed to target the tumor in vivo. The study suggested that the G5-PAMAM−6TAMRA−FA dendrimer had more retention ability to detect and accumulate tumor, and the fluorescence intensity had also increased in the tumor cells as compared to the G5-PAMAM−6TAMRA dendrimer (Thomas et al., 2004a; Wolinsky and Grinstaff, 2008).

8.4.11.1 Contrast agent for blood pool imaging

Gold nanoparticles have a great significance in CT as a contrast agent for blood pooling as they remain in blood circulation for a longer period of time, and they attenuate X-rays five to seven times higher than other iodine-based agents. To further increase their attenuation power, dendrimer-entrapped gold nanoparticles are being developed for imaging. Dendrimers contain an interior core that has the capacity to entrap gold nanoparticles. The peripherally placed amine groups can also mediate their entrapment by undergoing

modification when attached to different targeting agents. Thus dendrimers serve as an eminent platform in nanotechnology for developing dendrimer−gold nanoparticles that are multifunctional in nature. These types of nanoparticles have high stability pertaining to different conditions of pH and temperature (Tekade et al., 2015b).

The G5.0 and G6.0 dendrimers have been reported to serve as the most suitable contrast agents for blood pool imaging owing to their retaining capacity in blood circulation. G1.0 and G2.0 dendrimers easily permeate through blood vessels. G3.0 and G4.0 dendrimers are prone to undergo rapid excretion through kidneys; therefore they can be used as a contrast agent for imaging renal area (Wang et al., 2012).

8.4.11.2 *Modified bidentate dendrimer for bone imaging*

The structure of dendrimer was modified by incorporation of bidentate moiety such as iminodiacetate to develop a probe that has a high binding affinity toward bones. Hence, the imidoacetate modified poly-L-lysine dendrimer (IMPLD) was prepared through the convergent mode of synthesis. This newly constructed form of dendrimer served as a fluorescent imaging agent for bone. It was tagged with two fluorescent dyes such as cyanine and fluorescein. They were specifically linked to the dendrimer to determine their usage. The IMPLD purification was carried out through High Performance Liquid Chromatography (HPLC). This pure IMPLD along with the fluorescein was evaluated in vitro for determining its binding affinity toward biological calcium salts. The calcium salts present in the human body are calcium oxalate (CaO_x), calcium pyrophosphate (Py), hyaluronic acid (HA), calcium phosphate (Ph), and calcium carbonate ($CaCO_3$). The fluorescein-labeled IMPLD was incubated with the salts for 3 hours, and the absorbance of the supernatant was determined and compared with the original solution. It was observed that there was a decrease in the absorbance due to binding between IMPLD and the salt surface. Thus the increase in affinity of IMPLD for binding with the salt is indicated by a decrease in absorbance (Pes et al., 2017).

The percentage binding was calculated, and a graph was obtained as given in Fig. 8.36A. It was observed that IMPLD showed the highest percentage binding with CaO_x, that is, 93%, then 77% for Ph and 61% for HA. But it was also observed that it had a poor binding affinity toward Py and $CaCO_3$. Fig. 8.36B depicts the variable intensity of fluorescence in the supernatant of different salts. Fig. 8.36C indicates a visual determination of binding of IMPLD with the different calcium salts. To prove the potential of IMPLD as an imaging agent, the in vivo study was being carried out with the substitution of fluorescein with an appropriate near-infrared fluorescein dye, that is, cyanine 5.5 (Cy5.5-IMPLD) which was linked to IMPLD (Pes et al., 2017).

The Cy5.5-IMPLD formulation was given i.v. to the mice, and the images obtained demonstrated its rapid distribution in the entire body, and the probe was also eliminated through the kidneys rapidly (Fig. 8.37A). The fluorescence signal was detected in bones within a few hours, which was persistent for weeks. For example, on the fourth day an intense signal was detected in the knees and rib cage. Thus the maximum contrast was five times more than the background and the signal was retained in vivo for even more than 4 weeks (Fig. 8.37B). After removing the skin, the fluorescence imaging was performed ex vivo. Thus without the hindrance of skin, the images of ex vivo fluorescence depicted efficient details of bone structures such as the sternum, rib cage, vertebra, femur, and tail (Fig. 8.37C). This concluded that IMPLD has a good binding affinity toward the

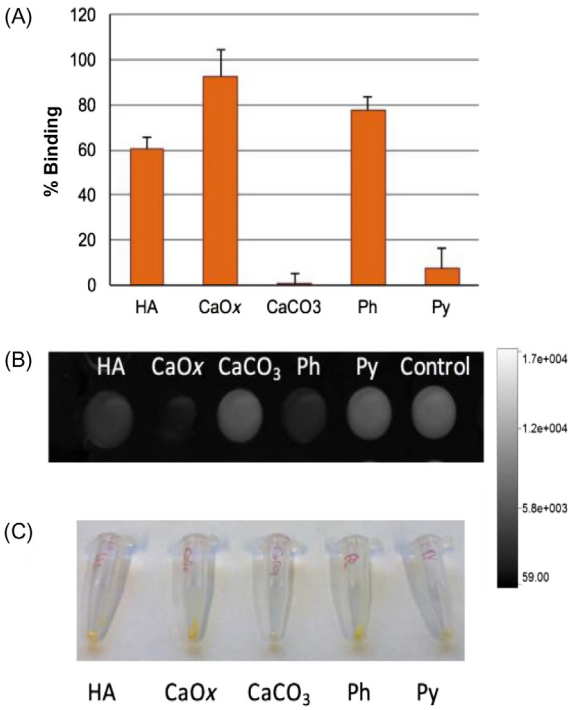

FIGURE 8.36 Comparison of IMPLD binding with different salts: (A) percentage binding of IMPLD; (B) fluorescence intensity of supernatant after 3 h of incubation, and (C) image of salts treated with IMPLD after washing. *IMPLD*, Imidoacetate modified poly-L-lysine dendrimer. Source*: Adapted with permission from Pes, L., Kim, Y., Tung, C.H., 2017. Bidentate iminodiacetate modified dendrimer for bone imaging. Bioorg. Med. Chem. Lett. 27 (5), 1252–1255.*

bone. Thereafter its stability was assessed by conducting IMPLD (0.1–50 μM) incubation with NIH3T3 fibroblasts. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cytotoxicity assay was also performed, which confirmed that IMPLD was nontoxic in that range of concentration. Thus IMPLD can be utilized for diagnosis of osteotropic tumors and also serve as a targeting ligand for mediating delivery of drugs employed for chemotherapy and hence provide therapeutic effectiveness for treating bone deformities (Pes et al., 2017).

8.4.12 Dendrimer as drug

Dendrimer has also been developed as agents for treating various diseases (Gajbhiye et al., 2009a); thus it possesses the characteristics of the drug molecule. Certain dendrimers of anionic nature have found to exhibit the antiviral effect. They have the affinity to bind to the viral surface and its components through electrostatic attraction; therefore they prevent the entry of the virus into a host cell and thus disrupt its further replication. Their activity is being tested against HIV virus including herpes simplex virus and respiratory syncytial virus (Gong et al., 2005). Cationic dendrimers have been studied to determine their antibiotic activity as they contain cationic functional groups such as lysine, which has the ability to interact with the bulky groups of the bacterial cell membrane that possesses a negative charge. Thus they change the permeability of the cell membrane and make it unstable ultimately leading to cell lysis (Klajnert et al., 2006).

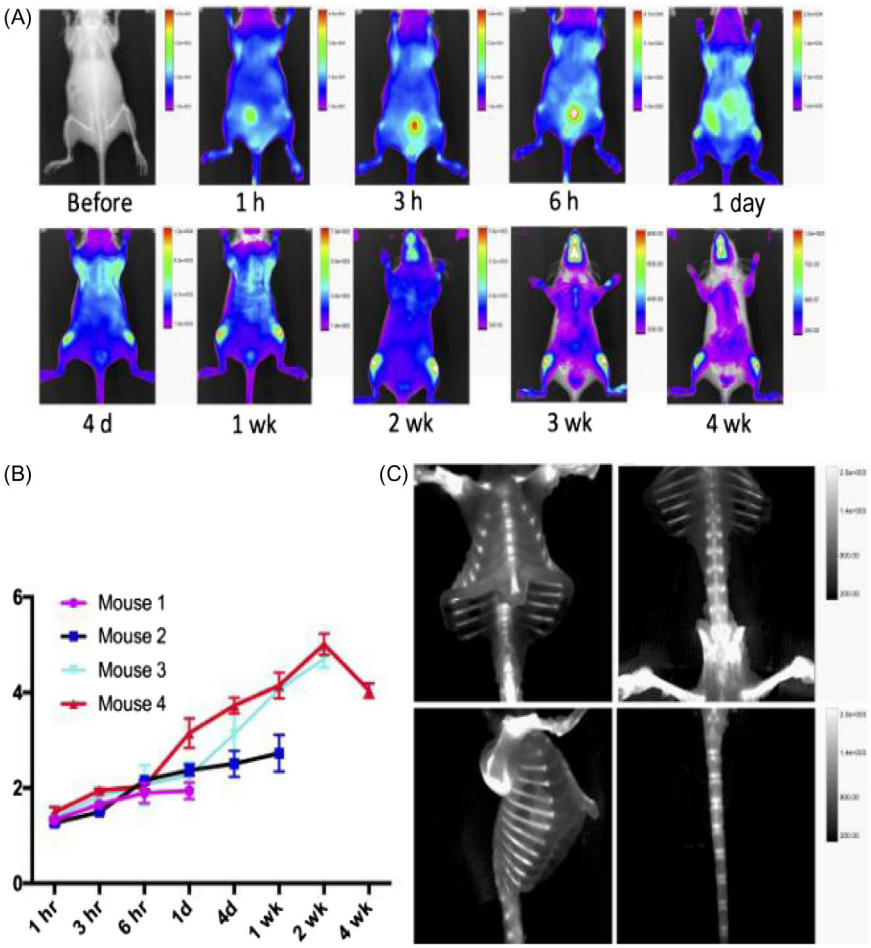

FIGURE 8.37 Images of in vivo and ex vivo fluorescence after i.v. injection of 20 nmol IMPLD with PBS. (A) In vivo optical images of anterior view at the indicated time points. The fluorescence of marked areas was measured. (B) Contrast ratios between the fluorescent intensity of knee and that of thigh muscle at different time points. (C) Ex vivo images after 2 weeks of injection of IMPLD depicting clear fluorescent signal in various bones. *IMPLD*, Imidoacetate modified poly-L-lysine dendrimer; *PBS*, phosphate buffer saline. Source*: Adapted with permission from Pes, L., Kim, Y., Tung, C.H., 2017. Bidentate iminodiacetate modified dendrimer for bone imaging. Bioorg. Med. Chem. Lett. 27 (5), 1252–1255.*

8.4.13 Nonmedical applications of dendrimers

8.4.13.1 Dendrimers as catalyst/enzymes

Dendrimers can be modified to act as a catalyst or enzyme. The dendritic core is being utilized for the attachment of catalytic groups to conduct the desired catalyst activity. An additional advantage provided by them is that of site specificity. The branches of dendrimers can be modulated to form several important moieties that serve as enzyme for carrying out enzymatic reactions. The shells of dendrimers are also being investigated to

produce a microenvironment that would favor catalysis or function as a protective barrier for the groups being incorporated in the core of a dendrimer. Raymond et al. developed the first catalytic peptide dendrimer to accelerate ester hydrolysis reaction. A recent example is that of PPI dendrimer containing imidazole groups that would act as a catalyst in the hydrolytic reaction of 2,4-dinitrophenylacetate in water, and also in Michael and nitroaldol reactions by reacting with phosphazene base. Metal ligand–based dendrimers are also used as a catalyst to conduct various reactions such as Diels–Alder, aldol, and organometallic couplings (Kofoed and Reymond, 2005).

8.4.13.2 Dendrimers for additives, printing inks, and paints

Dendritic polymers have recently grabbed attention in terms of their use as additives in printing inks, because they provide the adherent capacity to ink for binding to polar and nonpolar foils. Polyurethane paints containing dendritic polymers provide certain efficient properties such as chemical and physical resistance, increasing the intensity of light uptake, less weathering, fast speed, and impart good luster and glossy appearance, which can further be utilized in automobile and furniture industries. Polycarbonates are the best example of such dendritic polymers that can be used in inks and paints (Singh et al., 2014).

8.4.13.3 Dendrimers as separating agent

Dendrimers contain functional groups possessing high density and micelle-forming property, which provides an easy separation and recovery of the compound through ultrafiltration membrane; therefore they can perform the function of separating agents. The formed micelles then tend to increase the surface area of the particle by imparting its high density, which leads to its efficient separation; therefore the compound can be recovered or isolated through this approach. PAMAM dendrimers are being utilized in the separation of metal ions from waste water and soil, because they function as chelating agents, hence chelate the metal ions and remove them. PPI's and PAMAM dendrimers are further being worked upon to determine their potential as separating agent for the elimination of polycyclic aromatic hydrocarbon (Singh et al., 2014).

8.4.13.4 Industrial processes

The industrial applicability of dendrimers is attributed to their property of incorporating a wide variety of insoluble moieties, metal, etc. They also mediate the transfer of such molecules into their respective internal solvent. Fluorinated dendrimers have been reported to act as a solubilizing agent for supercritical CO_2. This approach can be utilized for the extraction of compounds in supercritical CO_2 and then highly efficient separation of hydrophilic compounds from water (Singh et al., 2014).

8.4.14 Dendrimer-based products

There are numerous types of dendrimers that are being utilized not only for research purposes but also for commercial applications. However, in the case of the pharmaceutical field the dendrimers are mostly employed which include Priostar, Astramol, PAMAM,

TABLE 8.2 Some important dendrimer-based products.

Brand name	Use	Company
Vivagel	Multiple antigen dendrimer for treating HIV infection	Starpharma
Stratus CS	Tecto-dendrimer that functions as a cardiac marker	Dade Behring
Alert Ticket	PAMAM dendrimer for anthrax detection	US Army Research Laboratory
Profect and Priostar	Tecto-dendrimer used for conducting targeted and therapeutic delivery to treat cancerous cells and diagnostic agent for the detection of cancer	Starpharma
Superfect	Amphiphilic type of dendrimer for gene transfection	Qiagen

HIV, Human immunodeficiency virus; *PAMAM*, polyamidoamine.

TABLE 8.3 Commercially available dendrimers and some dendrimer-based products under clinical trials.

Dendrimer	Brand name	Type of dendrimer	Company	Status
Commercially available dendrimer	Stratus CS	PAMAM	Dade behring	Marketed
	Priostar	PEHAM/PEA	Starpharma	Marketed
	Starburst	PAMAM	Starpharma	Marketed
	Alert ticket	PAMAM	U.S. Army Lab	Marketed
	Superfect	PAMAM	Qiagen	Marketed
	Polylysine	Poly-L-lysine	Starpharma	Marketed
	Priofect	PAMAM	Starpharma	Marketed
	Astramol	PPI	Starpharma	Marketed
Dendrimer products under clinical trials	Dendrimer-oxaliplatin	–	Starpharma	Preclinical
	Dendrimer-docetaxel	–	Starpharma	Preclinical
	Vivagel	Poly-L-lysine	Starpharma	Clinical trials (phase 3)

PAMAM, Polyamidoamine; *PEA*, poly(ester amine); *PEHAM*, poly(etherhydroxylamine); *PPI*, poly(propylene imine).

and Poly-l-lysine (PLL). In addition, dendrimer-based products are being widely explored by various research reports in both domains, that is, in the industry as well as in academic institutes. However, pharma companies have commercialized many dendrimer-based products, namely, SuperFect, VivaGel, Stratus CS, PrioFect, Priostar, and Alert Ticket. Some important commercially available dendrimer and dendrimer-based products under clinical trials are listed in Tables 8.2 and 8.3 (Noriega-Luna et al., 2014).

8.5 Conclusion

Dendrimers serve as an emerging platform for delivery of various moieties. Dendrimer-based carriers offer some noteworthy features, namely, versatile properties, uniformity in

size, low PDI, and molecular weight. Dendrimers are envisaged to be a promising polymer in the field of photodynamic therapy, biomedical, gene delivery, pharmaceutical, biopharmaceutical, siRNA delivery, oligonucleotide delivery, vaccine delivery, imaging in the 21st century. The inclusion of biodegradable properties in dendrimer can significantly increase its applicability. They consist of a core molecule, branches, and peripheral groups that can be synthesized by two techniques, namely, divergent growth method and convergent growth method. In addition, few recent methods have also been used for the development of dendrimers, for example, lego chemistry, click chemistry, double exponential growth method.

Moreover, versatility in the dendrimer structure makes it an ideal carrier. Further, easily controllable topographies of dendrimers, namely, size, shape, locking of liposomes in dendrimers, branching length, PEGylation, their surface functionality, and synthesis of targeted dendritic scaffolds as per the needs, make these systems as an ideal carrier in the numerous applications. Depending on different types of core and peripheral groups, the dendrimer can be classified as PAMAM dendrimer, PPI dendrimer, glycodendrimer, LC dendrimer, peptide dendrimer, etc. However, research is required in cost-effective synthesis, scaling up the synthesis procedure at an industrial scale, and to resolve certain toxicity issues. Eventually, by the improved synthesis techniques, further understanding of their distinctive features in drug delivery, and identification of new applications in several fields, dendrimers now become a potential candidate in drug delivery as well as in clinical applications.

Disclosures

There are no conflict of interests and disclosures associated with the manuscript.

Abbreviations

^{1}H NMR	Proton nuclear magnetic resonance
6TAMRA	6-carboxytetramethylrhodamine
BNCT	boron neutron capture therapy
$CaCO_3$	calcium carbonate
CaO_x	calcium oxalate
cDNA	complementary DNA
CT	computed tomography
EGF	epidermal growth factor
EPR	enhanced permeability and retention
DNA	deoxyribonucleic acid
EDA	ethylenediamine
FA	folic acid
FITC	fluorescein isothiocyanate
G	generation
Gd	gadolinium
HA	hyaluronic acid
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
IMPLD	imidoacetate modified poly-L-lysine dendrimer

KB cells	a subline of the ubiquitous KERATIN-forming tumor cell line HeLa
LC	liquid crystalline
LDH	lactate dehydrogenase
LLD	liposomal "locked in" dendrimer
MAP	multiple antigenic peptides
MRI	magnetic resonance imaging
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NA	nucleic acids
NOESY	nuclear Overhauser effect spectroscopy
ODNs	oligodeoxynucleotides
PAMAM	polyamidoamine
PAMAMOS	
PDI	polydispersity index
PEG	polyethylene glycol
PEO	polyethylene oxide
PGA	poly(L-glutamic acid)
PGDs	polyglycerol dendrimers
PGLSA	poly(glycerol−succinic acid)
Ph	calcium phosphate
PLL	poly-l-lysine
PPI	poly(propylene imine)
PSMA	prostate-specific membrane antigen
Py	calcium pyrophosphate
RBC	red blood cells
RGD	arginine−glycine−aspartic acid
siRNA	small interfering ribonucleic acid
SWCNT	single-walled carbon nanotubes
TEA	triethanolamine
THF	tetrahydrofuran
TMS	trimethylsilyl
TU-DTPA	thiourea diethylenetriaminepentaacetic acid

Acknowledgments

RKT would like to acknowledge Science and Engineering Research Board (Statutory Body Established Through an Act of Parliament: SERB Act 2008), Department of Science and Technology, Government of India, for a grant (Grant #ECR/2016/001964) and N-PDF funding (PDF/2016/003329) for work on targeted cancer therapy in Dr. Tekade's Laboaratory. Authors would also like to acknowledge the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, India, for supporting research on cancer and diabetes at NIPER—Ahmedabad.

References

Abbasi, E., Aval, S.F., Akbarzadeh, A., Milani, M., Nasrabadi, H.T., Joo, S.W., et al., 2014. Dendrimers: synthesis, applications, and properties. Nanoscale Res. Lett. 9 (1), 247.

Abedi-Gaballu, F., Dehghan, G., Ghaffari, M., Yekta, R., Abbaspour-Ravasjani, S., Baradaran, B., et al., 2018. PAMAM dendrimers as efficient drug and gene delivery nanosystems for cancer therapy. Appl. Mater. Today 12, 177−190.

Agarwal, A., Gupta, U., Asthana, A., Jain, N.K., 2009. Dextran conjugated dendritic nanoconstructs as potential vectors for anti-cancer agent. Biomaterials 30 (21), 3588−3596.

Al-Jamal, K.T., Ramaswamy, C., Florence, A.T., 2005. Supramolecular structures from dendrons and dendrimers. Adv. Drug Deliv. Rev. 57 (15), 2238−2270.

Arima, H., Kihara, F., Hirayama, F., Uekama, K., 2001. Enhancement of gene expression by polyamidoamine dendrimer conjugates with alpha-, beta-, and gamma-cyclodextrins. Bioconjug. Chem. 12 (4), 476–484.
Asthana, A., Chauhan, A.S., Diwan, P.V., Jain, N.K., 2005. Poly(amidoamine) (PAMAM) dendritic nanostructures for controlled site-specific delivery of acidic anti-inflammatory active ingredient. AAPS PharmSciTech 6 (3), E536–E542.
Baek, M.-G., Roy, R., 2002. Synthesis and protein binding properties of T-antigen containing GlycoPAMAM dendrimers. Bioorg. Med. Chem. 10, 11–17.
Bayele, H.K., Ramaswamy, C., Wilderspin, A.F., Srai, K.S., Toth, I., Florence, A.T., 2006. Protein transduction by lipidic peptide dendrimers. J. Pharm. Sci. 95 (6), 1227–1237.
Betley, T.A., Hessler, J.A., Mecke, A., Banaszak Holl, M.M., Orr, B.G., Uppuluri, S., et al., 2002. Tapping mode atomic force microscopy investigation of poly(amidoamine) core–shell tecto(dendrimers) using carbon nanoprobes. Langmuir 18 (8), 3127–3133.
Bhadra, D., Bhadra, S., Jain, P., Jain, N.K., 2002. Pegnology: a review of PEG-ylated systems. Pharmazie 57 (1), 5–29.
Bielinska, A., Kukowska-Latallo, J.F., Johnson, J., Tomalia, D.A., Baker Jr, J.R., 1996. Regulation of in vitro gene expression using antisense oligonucleotides or antisense expression plasmids transfected using starburst PAMAM dendrimers. Nucleic Acids Res. 24 (11), 2176–2182.
Chen, W., Turro, N.J., Tomalia, D.A., 2000. Using ethidium bromide to probe the interactions between DNA and dendrimers. Langmuir 16 (1), 15–19.
Chen, H.T., Neerman, M.F., Parrish, A.R., Simanek, E.E., 2004. Cytotoxicity, hemolysis, and acute in vivo toxicity of dendrimers based on melamine, candidate vehicles for drug delivery. J. Am. Chem. Soc. 126 (32), 10044–10048.
Choe, Y.H., Conover, C.D., Wu, D., Royzen, M., Gervacio, Y., Borowski, V., et al., 2002. Anticancer drug delivery systems: multi-loaded *N*4-acyl poly(ethylene glycol) prodrugs of ara-C. II. Efficacy in ascites and solid tumors. J. Control. Release 79 (1–3), 55–70.
Choi, Y., Baker Jr., J.R., 2005. Targeting cancer cells with DNA-assembled dendrimers: a mix and match strategy for cancer. Cell Cycle 4 (5), 669–671.
Choudhary, S., Gupta, L., Rani, S., Dave, K., Gupta, U., 2017. Impact of dendrimers on solubility of hydrophobic drug molecules. Front. Pharmacol. 8, 261.
Córdoba, E.V., Pion, M., Rasines, B., Filippini, D., Komber, H., Ionov, M., et al., 2013. Glycodendrimers as new tools in the search for effective anti-HIV DC-based immunotherapies. Nanomedicine 9 (7), 972–984.
Cui, Y., Liang, B., Wang, L., Zhu, L., Kang, J., Sun, H., et al., 2018. Enhanced biocompatibility of PAMAM dendrimers benefiting from tuning their surface charges. Mater. Sci. Eng., C: Mater. Biol. Appl. 93, 332–340.
D'emanuele, A., Attwood, D., 2005. Dendrimer–drug interactions. Adv. Drug Deliv. Rev. 57 (15), 2147–2162.
Desgrosellier, J.S., Cheresh, D.A., 2010. Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer 10 (1), 9–22.
Dhakad, R.S., Tekade, R.K., Jain, N.K., 2013. Cancer targeting potential of folate targeted nanocarrier under comparative influence of tretinoin and dexamethasone. Curr Drug Deliv 10 (4), 477–491.
Domb, A., Barenholz, Y., Eliyahu, H., 2005. Polymers for DNA delivery. Molecules 10 (1), 34–64.
Duncan, R., Izzo, L., 2005. Dendrimer biocompatibility and toxicity. Adv. Drug Deliv. Rev. 57 (15), 2215–2237.
Dvornic, P.R., de Leuze-Jallouli, A.M., Owen, M.J., Perz, S.V., 2000. Radially layered poly(amidoamine-organosilicon) dendrimers. Macromolecules 33 (15), 5366–5378.
Dwivedi, N., Shah, J., Mishra, V., Mohd Amin, M.C., Iyer, A.K., Tekade, R.K., Kesharwani, P., 2016. Dendrimer-mediated approaches for the treatment of brain tumor. J Biomater Sci Polym Ed 27 (7), 557–580.
Favre, D., Provost, N., Blouin, V., Blancho, G., Cherel, Y., Salvetti, A., et al., 2001. Immediate and long-term safety of recombinant adeno-associated virus injection into the nonhuman primate muscle. Mol. Ther. 4 (6), 559–566.
Fox, L.J., Richardson, R.M., Briscoe, W.H., 2018. PAMAM dendrimer—cell membrane interactions. Adv. Colloid Interface Sci. 257, 1–18.
Gajbhiye, V., Palanirajan, V.K., Tekade, R.K., Jain, N.K., 2009a. Dendrimers as therapeutic agents: a systematic review. J Pharm Pharmacol 61 (8), 989–1003.
Gajbhiye, V., Vijayaraj Kumar, P., Tekade, R.K., Jain, N.K., 2009b. PEGylated PPI dendritic architectures for sustained delivery of H2 receptor antagonist. Eur J Med Chem 44 (3), 1155–1166.
Gajbhiye, V., Ganesh, N., Barve, J., Jain, N.K., 2013. Synthesis, characterization and targeting potential of zidovudine loaded sialic acid conjugated-mannosylated poly(propyleneimine) dendrimers. Eur. J. Pharm. Sci. 48 (4–5), 668–679.

Gardikis, K., Hatziantoniou, S., Bucos, M., Fessas, D., Signorelli, M., Felekis, T., et al., 2010. New drug delivery nanosystem combining liposomal and dendrimeric technology (liposomal locked-in dendrimers) for cancer therapy. J. Pharm. Sci. 99 (8), 3561–3571.
Gebhart, C.L., Kabanov, A.V., 2001. Evaluation of polyplexes as gene transfer agents. J. Control. Release 73 (2–3), 401–416.
Ghaffari, M., Dehghan, G., Abedi-Gaballu, F., Kashanian, S., Baradaran, B., Dolatabadi, J.E.N., et al., 2018. Surface functionalized dendrimers as controlled-release delivery nanosystems for tumor targeting. Eur. J. Pharm. Sci. 122, 311–330.
Gong, E., Matthews, B., McCarthy, T., Chu, J., Holan, G., Raff, J., et al., 2005. Evaluation of dendrimer SPL7013, a lead microbicide candidate against herpes simplex viruses. Antiviral Res. 68 (3), 139–146.
Gorain, B., Tekade, M., Kesharwani, P., Iyer, A.K., Kalia, K., Tekade, R.K., 2017. The use of nanoscaffolds and dendrimers in tissue engineering. Drug Discov. Today 22 (4), 652–664.
Gorkhe, Y., Pande, V., Autade, R., 2018. Dendrimers: a platform for novel drug delivery system. Int. J. Pharm. Drug Anal. 6 (4), 439–445.
Gorzkiewicz, M., Jatczak-Pawlik, I., Studzian, M., Pulaski, L., Appelhans, D., Voit, B., et al., 2018. Glycodendrimer nanocarriers for direct delivery of fludarabine triphosphate to leukemic cells: improved pharmacokinetics and pharmacodynamics of fludarabine. Biomacromolecules 19 (2), 531–543.
Guillon, D., Deschenaux, R., 2002. Liquid-crystalline dendrimers. Curr. Opin. Solid State Mater. Sci. 6 (6), 515–525.
Guillot-Nieckowski, M., Joester, D., Stohr, M., Losson, M., Adrian, M., Wagner, B., et al., 2007. Self-assembly, DNA complexation, and pH response of amphiphilic dendrimers for gene transfection. Langmuir 23 (2), 737–746.
Gupta, U., Agashe, H.B., Jain, N.K., 2007. Polypropylene imine dendrimer mediated solubility enhancement: effect of pH and functional groups of hydrophobes. J. Pharm. Pharm. Sci. 10 (3), 358–367.
Hu, J., Hu, K., Cheng, Y., 2016. Tailoring the dendrimer core for efficient gene delivery. Acta Biomater. 35, 1–11.
Huang, D., Wu, D., 2018. Biodegradable dendrimers for drug delivery. Mater. Sci. Eng., C: Mater. Biol. Appl. 90, 713–727.
Hussain, M., Shchepinov, M., Sohail, M., Benter, I.F., Hollins, A.J., Southern, E.M., et al., 2004. A novel anionic dendrimer for improved cellular delivery of antisense oligonucleotides. J. Control. Release 99 (1), 139–155.
Jain, R.K., 1987. Transport of molecules in the tumor interstitium: a review. Cancer Res. 47 (12), 3039–3051.
Jain, N.K., Tekade, R.K., 2013. Dendrimers for enhanced drug solubilization. In: Douroumis, D., Fahr, A. (Eds.), Drug Delivery Strategies for Poorly Water-Soluble Drugs, I st edn. John Wiley & Sons, Ltd., pp. 373–409.
Jain, K., Kesharwani, P., Gupta, U., Jain, N.K., 2010. Dendrimer toxicity: let's meet the challenge. Int. J. Pharm. 394 (1–2), 122–142.
Jain, S., Kesharwani, P., Tekade, R.K., Jain, N.K., 2015. One platform comparison of solubilization potential of dendrimer with some solubilizing agents. Drug Dev Ind Pharm 41 (5), 722–727.
Jevprasesphant, R., Penny, J., Attwood, D., McKeown, N.B., D'Emanuele, A., 2003. Engineering of dendrimer surfaces to enhance transepithelial transport and reduce cytotoxicity. Pharm. Res. 20 (10), 1543–1550.
Jin, X., Zhou, L., Zhu, B., Jiang, X., Zhu, N., 2018. Silver-dendrimer nanocomposites as oligonucleotide labels for electrochemical stripping detection of DNA hybridization. Biosens. Bioelectron. 107, 237–243.
Joester, D., Losson, M., Pugin, R., Heinzelmann, H., Walter, E., Merkle, H.P., et al., 2003. Amphiphilic dendrimers: novel self-assembling vectors for efficient gene delivery. Angew. Chem. Int. Ed. Engl. 42 (13), 1486–1490.
Kaminskas, L.M., Kelly, B.D., McLeod, V.M., Sberna, G., Owen, D.J., Boyd, B.J., et al., 2011. Characterisation and tumour targeting of PEGylated polylysine dendrimers bearing doxorubicin via a pH labile linker. J. Control. Release 152 (2), 241–248.
Kawaguchi, T., Walker, K.L., Wilkins, C.L., Moore, J.S., 1995. Double exponential dendrimer growth. J. Am. Chem. Soc. 117 (8), 2159–2165.
Kay, M.A., Glorioso, J.C., Naldini, L., 2001. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat. Med. 7 (1), 33–40.
Kesharwani, P., Gajbhiye, V., Tekade, R.K., Jain, N.K., 2011a. Evaluation of dendrimer safety and efficacy through cell line studies. Curr Drug Targets 12 (10), 1478–1497.
Kesharwani, P., Tekade, R.K., Gajbhiye, V., Jain, K., Jain, N.K., 2011b. Cancer targeting potential of some ligand-anchored poly(propylene imine) dendrimers: a comparison. Nanomedicine 7 (3), 295–304.

Kesharwani, P., Tekade, R.K., Jain, N.K., 2014a. Formulation development and in vitro-in vivo assessment of the fourth-generation PPI dendrimer as a cancer-targeting vector. Nanomedicine (Lond) 9 (15), 2291–2308.
Kesharwani, P., Tekade, R.K., Jain, N.K., 2015a. Dendrimer generational nomenclature: the need to harmonize. Drug Discov Today 20 (5), 497–499.
Kesharwani, P., Tekade, R.K., Jain, N.K., 2015b. Generation dependent safety and efficacy of folic acid conjugated dendrimer based anticancer drug formulations. Pharm Res 32 (4), 1438–1450.
Kesharwani, P., Amin, M.C.I.M., Giri, N., Jain, A., Gajbhiye, V., 2017. Dendrimers in targeting and delivery of drugs. In: Mishra, V., Kesharwani, P., Mohd Amin, M.C.I., Iyer, A. (Eds.), Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes. Academic Press, pp. 363–388.
Kim, S., Kim, J.H., Jeon, O., Kwon, I.C., Park, K., 2009. Engineered polymers for advanced drug delivery. Eur. J. Pharm. Biopharm. 71 (3), 420–430.
Kim, Y.M., Park, S.C., Jang, M.K., 2017. Targeted gene delivery of polyethyleneimine-grafted chitosan with RGD dendrimer peptide in alphavbeta3 integrin-overexpressing tumor cells. Carbohydr. Polym. 174, 1059–1068.
Klajnert, B., Janiszewska, J., Urbanczyk-Lipkowska, Z., Bryszewska, M., Shcharbin, D., Labieniec, M., 2006. Biological properties of low molecular mass peptide dendrimers. Int. J. Pharm. 309 (1–2), 208–217.
Klajnert, B., Appelhans, D., Komber, H., Morgner, N., Schwarz, S., Richter, S., et al., 2008. The influence of densely organized maltose shells on the biological properties of poly(propylene imine) dendrimers: new effects dependent on hydrogen bonding. Chemistry 14 (23), 7030–7041.
Kofoed, J., Reymond, J.-L., 2005. Dendrimers as artificial enzymes. Curr. Opin. Chem. Biol. 9 (6), 656–664.
Kojima, C., Kono, K., Maruyama, K., Takagishi, T., 2000. Synthesis of polyamidoamine dendrimers having poly (ethylene glycol) grafts and their ability to encapsulate anticancer drugs. Bioconjug. Chem. 11 (6), 910–917.
Kojima, C., Toi, Y., Harada, A., Kono, K., 2007. Preparation of poly(ethylene glycol)-attached dendrimers encapsulating photosensitizers for application to photodynamic therapy. Bioconjug. Chem. 18 (3), 663–670.
Kojima, C., Turkbey, B., Ogawa, M., Bernardo, M., Regino, C.A., Bryant, L.H., et al., 2011. Dendrimer-based MRI contrast agents: the effects of PEGylation on relaxivity and pharmacokinetics. Nanomedicine 7 (6), 1001–1008.
Kolb, H.C., Sharpless, K.B., 2003. The growing impact of click chemistry on drug discovery. Drug Discov. Today 8 (24), 1128–1137.
Li, C., 2002. Poly(L-glutamic acid)—anticancer drug conjugates. Adv. Drug Deliv. Rev. 54 (5), 695–713.
Li, C., Yu, D.F., Newman, R.A., Cabral, F., Stephens, L.C., Hunter, N., et al., 1998. Complete regression of well-established tumors using a novel water-soluble poly(L-glutamic acid)-paclitaxel conjugate. Cancer Res. 58 (11), 2404–2409.
Li, D., Fan, Y., Shen, M., Bányai, I., Shi, X., 2019. Design of dual drug-loaded dendrimer/carbon dot nanohybrids for fluorescence imaging and enhanced chemotherapy of cancer cells. J. Mater. Chem. B 7 (2), 277–285.
Lim, J., Simanek, E.E., 2012. Triazine dendrimers as drug delivery systems: from synthesis to therapy. Adv. Drug Deliv. Rev. 64 (9), 826–835.
Liu, H., Wang, Y., Wang, M., Xiao, J., Cheng, Y., 2014. Fluorinated poly(propylenimine) dendrimers as gene vectors. Biomaterials 35 (20), 5407–5413.
Lo, S.T., Kumar, A., Hsieh, J.T., Sun, X., 2013. Dendrimer nanoscaffolds for potential theranostics of prostate cancer with a focus on radiochemistry. Mol. Pharm. 10 (3), 793–812.
Loup, C., Zanta, M.A., Caminade, A.M., Majoral, J.P., Meunier, B., 1999. Preparation of water-soluble cationic phosphorus-containing dendrimers as DNA transfecting agents. Chem.—Eur. J. 5 (12), 3644–3650.
Luo, D., Haverstick, K., Belcheva, N., Han, E., Saltzman, W.M., 2002. Poly (ethylene glycol)-conjugated PAMAM dendrimer for biocompatible, high-efficiency DNA delivery. Macromolecules 35 (9), 3456–3462.
Luo, K., Li, C., Li, L., She, W., Wang, G., Gu, Z., 2012. Arginine functionalized peptide dendrimers as potential gene delivery vehicles. Biomaterials 33 (19), 4917–4927.
Madaan, K., Kumar, S., Poonia, N., Lather, V., Pandita, D., 2014. Dendrimers in drug delivery and targeting: drug-dendrimer interactions and toxicity issues. J. Pharm. Bioallied Sci. 6 (3), 139–150.
Malik, N., Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J., et al., 2000a. Dendrimers:: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. J. Control. Release 65 (1–2), 133–148.
Malik, N., Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J., et al., 2000b. Erratum: dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of

125I-labelled polyamidoamine dendrimers in vivo (Journal of Controlled Release 65 (2000)(133-148)). J. Control. Release 68 (2), 299–302.
Malik, N., Duncan, R., Tomalia, D.A., Esfand, R., 2003. An Antineoplastic Dendritic Polymer Drug Delivery System, WO03037383.
Manikkath, J., Hegde, A.R., Kalthur, G., Parekh, H.S., Mutalik, S., 2017. Influence of peptide dendrimers and sonophoresis on the transdermal delivery of ketoprofen. Int. J. Pharm. 521 (1–2), 110–119.
Markatou, E., Gionis, V., Chryssikos, G.D., Hatziantoniou, S., Georgopoulos, A., Demetzos, C., 2007. Molecular interactions between dimethoxycurcumin and Pamam dendrimer carriers. Int. J. Pharm. 339 (1–2), 231–236.
Markowicz-Piasecka, M., Mikiciuk-Olasik, E., 2016. Dendrimers in drug delivery. In: Grumezescu, A.M. (Ed.), Nanobiomaterials in Drug Delivery. William Andrew Publishing, pp. 39–74.
Milhem, O., Myles, C., McKeown, N., Attwood, D., D'Emanuele, A., 2000. Polyamidoamine Starburst® dendrimers as solubility enhancers. Int. J. Pharm. 197 (1–2), 239–241.
Militello, M.P., Hernández-Ramírez, R.E., Lijanova, I.V., Previtali, C.M., Bertolotti, S.G., Arbeloa, E.M., 2018. Novel PAMAM dendrimers with porphyrin core as potential photosensitizers for PDT applications. J. Photochem. Photobiol., A: Chem. 353, 71–76.
Mody, N., Tekade, R.K., Mehra, N.K., Chopdey, P., Jain, N.K., 2014. Dendrimer, liposomes, carbon nanotubes and PLGA nanoparticles: one platform assessment of drug delivery potential. AAPS PharmSciTech 15 (2), 388–399.
Mukherjee, S.P., Davoren, M., Byrne, H.J., 2010. In vitro mammalian cytotoxicological study of PAMAM dendrimers–towards quantitative structure activity relationships. Toxicol. In Vitro 24 (1), 169–177.
Muniswamy, V.J., Raval, N., Gondaliya, P., Tambe, V., Kalia, K., Tekade, R.K., 2019. 'Dendrimer-Cationized-Albumin' encrusted polymeric nanoparticle improves BBB penetration and anticancer activity of doxorubicin. Int J Pharm 555, 77–99.
Nanjwade, B.K., Bechra, H.M., Derkar, G.K., Manvi, F.V., Nanjwade, V.K., 2009. Dendrimers: emerging polymers for drug-delivery systems. Eur. J. Pharm. Sci. 38 (3), 185–196.
Navarro, G., Tros de Ilarduya, C., 2009. Activated and non-activated PAMAM dendrimers for gene delivery in vitro and in vivo. Nanomedicine 5 (3), 287–297.
Noriega-Luna, B., Godínez, L.A., Rodríguez, F.J., Rodríguez, A., Larrea, G., Sosa-Ferreyra, C., et al., 2014. Applications of dendrimers in drug delivery agents, diagnosis, therapy, and detection. J. Nanomater. 2014, 39.
Okuda, T., Kawakami, S., Maeie, T., Niidome, T., Yamashita, F., Hashida, M., 2006. Biodistribution characteristics of amino acid dendrimers and their PEGylated derivatives after intravenous administration. J. Control. Release 114 (1), 69–77.
Ooya, T., Lee, J., Park, K., 2003. Effects of ethylene glycol-based graft, star-shaped, and dendritic polymers on solubilization and controlled release of paclitaxel. J. Control. Release 93 (2), 121–127.
Ooya, T., Lee, J., Park, K., 2004. Hydrotropic dendrimers of generations 4 and 5: synthesis, characterization, and hydrotropic solubilization of paclitaxel. Bioconjug. Chem. 15 (6), 1221–1229.
Öztürk, K., Esendağlı, G., Gürbüz, M.U., Tülü, M., Çalış, S., 2017. Effective targeting of gemcitabine to pancreatic cancer through PEG-cored Flt-1 antibody-conjugated dendrimers. Int. J. Pharm. 517 (1–2), 157–167.
Padilla De Jesús, O.L., Ihre, H.R., Gagne, L., Fréchet, J.M., Szoka, F.C., 2002. Polyester dendritic systems for drug delivery applications: in vitro and in vivo evaluation. Bioconjug. Chem. 13 (3), 453–461.
Pandey, P.K., Maheshwari, R., Raval, N., Gondaliya, P., Kalia, K., Tekade, R.K., 2019. Nanogold-core multifunctional dendrimer for pulsatile chemo-, photothermal- and photodynamic- therapy of rheumatoid arthritis. J Colloid Interface Sci 544, 61–77.
Pandi, P., Jain, A., Kommineni, N., Ionov, M., Bryszewska, M., Khan, W., 2018. Dendrimer as a new potential carrier for topical delivery of siRNA: a comparative study of dendriplex vs. lipoplex for delivery of TNF-alpha siRNA. Int. J. Pharm. 550 (1–2), 240–250.
Papagiannaros, A., Dimas, K., Papaioannou, G.T., Demetzos, C., 2005. Doxorubicin-PAMAM dendrimer complex attached to liposomes: cytotoxic studies against human cancer cell lines. Int. J. Pharm. 302 (1–2), 29–38.
Parajapati, S.K., Maurya, S.D., Das, M.K., Tilak, V.K., Verma, K.K., Dhakar, R.C., 2016. Potential application of dendrimers in drug delivery: a concise review and update. J. Drug Deliv. Ther. 6 (2), 71–88.
Patel, H.K., Gajbhiye, V., Kesharwani, P., Jain, N.K., 2016. Ligand anchored poly(propyleneimine) dendrimers for brain targeting: comparative in vitro and in vivo assessment. J. Colloid Interface Sci. 482, 142–150.

Patri, A.K., Majoros, I.J., Baker, J.R., 2002. Dendritic polymer macromolecular carriers for drug delivery. Curr. Opin. Chem. Biol. 6 (4), 466–471.
Patri, A.K., Myc, A., Beals, J., Thomas, T.P., Bander, N.H., Baker Jr., J.R., 2004. Synthesis and in vitro testing of J591 antibody-dendrimer conjugates for targeted prostate cancer therapy. Bioconjug. Chem. 15 (6), 1174–1181.
Patri, A.K., Kukowska-Latallo, J.F., Baker Jr., J.R., 2005. Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex. Adv. Drug Deliv. Rev. 57 (15), 2203–2214.
Pearson, R.M., Hsu, H.-J., Bugno, J., Hong, S., 2014. Understanding nano-bio interactions to improve nanocarriers for drug delivery. MRS Bull. 39 (3), 227–237.
Pedziwiatr-Werbicka, E., Ferenc, M., Zaborski, M., Gabara, B., Klajnert, B., Bryszewska, M., 2011. Characterization of complexes formed by polypropylene imine dendrimers and anti-HIV oligonucleotides. Colloids Surf., B: Biointerfaces 83 (2), 360–366.
Pes, L., Kim, Y., Tung, C.H., 2017. Bidentate iminodiacetate modified dendrimer for bone imaging. Bioorg. Med. Chem. Lett. 27 (5), 1252–1255.
Ponomarenko, S., Boiko, N., Shibaev, V., 2001. Liquid-crystalline dendrimers. Polym. Sci. Ser. C 43 (1), 1–45.
Pospisil, M., Vannucci, L., Horvath, O., Fiserova, A., Krausova, K., Bezouska, K., et al., 2000. Cancer immunomodulation by carbohydrate ligands for the lymphocyte receptor NKR-P1. Int. J. Oncol. 16 (2), 267–276.
Pospisil, M., Vannucci, L., Fiserova, A., Krausova, K., Horvath, O., Kren, V., et al., 2001. Glycodendrimeric ligands of c-type lectin receptors as therapeutic agents in experimental cancer. Adv. Exp. Med. Biol. 495, 343–347.
Praetorius, M., Pfannenstiel, S., Klingmann, C., Baumann, I., Plinkert, P.K., Staecker, H., 2008. Expression patterns of non-viral transfection with GFP in the organ of Corti in vitro and in vivo. Gene therapy of the inner ear with non-viral vectors. HNO 56 (5), 524–529.
Prajapati, R.N., Tekade, R.K., Gupta, U., Gajbhiye, V., Jain, N.K., 2009. Dendimer-mediated solubilization, formulation development and in vitro-in vivo assessment of piroxicam. Mol Pharm 6 (3), 940–950.
Pu, L., 2003. Synthesis and study of binaphthyl-based chiral dendrimers. J. Photochem. Photobiol. A: Chem. 155 (1–3), 47–55.
Purohit, G., Sakthivel, T., Florence, A.T., 2001. Interaction of cationic partial dendrimers with charged and neutral liposomes. Int. J. Pharm. 214 (1–2), 71–76.
Quintana, S., García, M.Á., Marina, M.L., Gómez, R., de la Mata, F.J., Ortega, P.J., 2017. Synthesis of chiral carbosilane dendrimers with l-cysteine and *N*-acetyl-l-cysteine on their surface and their application as chiral selectors for enantiomer separation by capillary electrophoresis. Tetrahedron: Asymmetry 28 (12), 1797–1802.
Raval, N., Maheshwari, R., Kalia, K., Tekade, R.K., 2019. Fabrication of Mucoadhesive-Dendrimers as Solid Dosage Forms. Methods Mol Biol 2000, 93–109.
Roy, R., Baek, M.-G., Rittenhouse-Olson, K., 2001. Synthesis of *N*,*N*′-bis(acrylamido)acetic acid-based T-antigen glycodendrimers and their mouse monoclonal IgG antibody binding properties. J. Am. Chem. Soc. 123 (9), 1809–1816.
Sadekar, S., Ghandehari, H., 2012. Transepithelial transport and toxicity of PAMAM dendrimers: implications for oral drug delivery. Adv. Drug Deliv. Rev. 64 (6), 571–588.
Sadler, K., Tam, J.P., 2002. Peptide dendrimers: applications and synthesis. J. Biotechnol. 90 (3–4), 195–229.
Santhakumaran, L.M., Thomas, T., Thomas, T.J., 2004. Enhanced cellular uptake of a triplex-forming oligonucleotide by nanoparticle formation in the presence of polypropylenimine dendrimers. Nucleic Acids Res. 32 (7), 2102–2112.
Selin, M., Peltonen, L., Hirvonen, J., Bimbo, L.M., 2016. Dendrimers and their supramolecular nanostructures for biomedical applications. J. Drug Deliv. Sci. Technol. 34, 10–20.
Serkova, E.S., Krasnova, I.Y., Milenin, S.A., Selezneva, E.V., Tatarinova, E.A., Boldyrev, K.L., et al., 2018. Core/shell hybrid dendrimers: controllable rigidity determines molecular behaviour. Polymer 138, 83–91.
Shamis, M., Lode, H.N., Shabat, D., 2004. Bioactivation of self-immolative dendritic prodrugs by catalytic antibody 38C2. J. Am. Chem. Soc. 126 (6), 1726–1731.
Sharma, A.K., Gothwal, A., Kesharwani, P., Alsaab, H., Iyer, A.K., Gupta, U., 2017. Dendrimer nanoarchitectures for cancer diagnosis and anticancer drug delivery. Drug Discov. Today 22 (2), 314–326.
Shaunak, S., Thomas, S., Gianasi, E., Godwin, A., Jones, E., Teo, I., et al., 2004. Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. Nat. Biotechnol. 22 (8), 977–984.

Sherje, A.P., Jadhav, M., Dravyakar, B.R., Kadam, D., 2018. Dendrimers: a versatile nanocarrier for drug delivery and targeting. Int. J. Pharm. 548 (1), 707–720.
Shukla, R., Thomas, T.P., Peters, J., Kotlyar, A., Myc, A., Baker Jr., J.R., 2005. Tumor angiogenic vasculature targeting with PAMAM dendrimer-RGD conjugates. Chem. Commun. (Cambridge, England) 46, 5739–5741.
Singh, U., Dar, M.M., Hashmi, A.A., 2014. Dendrimers: synthetic strategies, properties and applications. Orient. J. Chem. 30 (3), 911–922.
Somani, S., Laskar, P., Altwaijry, N., Kewcharoenvong, P., Irving, C., Robb, G., et al., 2018. PEGylation of polypropylenimine dendrimers: effects on cytotoxicity, DNA condensation, gene delivery and expression in cancer cells. Sci. Rep. 8, 9410.
Soni, N., Tekade, M., Kesharwani, P., Bhattacharya, P., Maheshwari, R., Dua, K., Hansbro, P.M., Tekade, R.K., 2017. Recent Advances in Oncological Submissions of Dendrimer. Curr Pharm Des 23 (21), 3084–3098.
Svenningsen, S.W., Janaszewska, A., Ficker, M., Petersen, J.F., Klajnert-Maculewicz, B., Christensen, J.B., 2016. Two for the price of one: PAMAM-dendrimers with mixed phosphoryl choline and oligomeric poly(caprolactone) surfaces. Bioconjug. Chem. 27 (6), 1547–1557.
Svenson, S., Chauhan, A., 2008. Dendrimers for enhanced drug solubilization. Nanomedicine (Lond.) 3, 679–702.
Tam, J.P., 1988. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc. Natl. Acad. Sci. U.S.A. 85 (15), 5409–5413.
Tambe, V., Thakkar, S., Raval, N., Sharma, D., Kalia, K., Tekade, R.K., 2017. Surface engineered dendrimers in siRNA delivery and gene silencing. Curr. Pharm. Des. 23 (20), 2952–2975.
Tang, M.X., Redemann, C.T., Szoka Jr., F.C., 1996. In vitro gene delivery by degraded polyamidoamine dendrimers. Bioconjug. Chem. 7 (6), 703–714.
Tansey, W., Ke, S., Cao, X.Y., Pasuelo, M.J., Wallace, S., Li, C., 2004. Synthesis and characterization of branched poly(L-glutamic acid) as a biodegradable drug carrier. J. Control. Release 94 (1), 39–51.
Tekade, R.K., Kumar, P.V., Jain, N.K., 2008a. Dendrimers in oncology: an expanding horizon. Chem. Rev. 109 (1), 49–87.
Tekade, R.K., Dutta, T., Tyagi, A., Bharti, A.C., Das, B.C., Jain, N.K., 2008b. Surface-engineered dendrimers for dual drug delivery: a receptor up-regulation and enhanced cancer targeting strategy. J Drug Target 16 (10), 758–772.
Tekade, R.K., Kumar, P.V., Jain, N.K., 2009. Dendrimers in oncology: an expanding horizon. Chem. Rev. 109 (1), 49–87.
Tekade, R.K., Dutta, T., Gajbhiye, V., Jain, N.K., 2009a. Exploring dendrimer towards dual drug delivery: pH responsive simultaneous drug-release kinetics. J Microencapsul 26 (4), 287–296.
Tekade, R.K., Kumar, P.V., Jain, N.K., 2009b. Dendrimers in oncology: an expanding horizon. Chem Rev 109 (1), 49–87.
Tekade, R.K., Maheshwari, R.G., Sharma, P.A., Tekade, M., Chauhan, A.S., 2015a. siRNA Therapy, Challenges and Underlying Perspectives of Dendrimer as Delivery Vector. Curr Pharm Des 21 (31), 4614–4636.
Tekade, R.K., Tekade, M., Kumar, M., Chauhan, A.S., 2015b. Dendrimer-stabilized smart-nanoparticle (DSSN) platform for targeted delivery of hydrophobic antitumor therapeutics. Pharm Res 32 (3), 910–928.
Thomas, T.P., Myaing, M.T., Ye, J.Y., Candido, K., Kotlyar, A., Beals, J., et al., 2004a. Detection and analysis of tumor fluorescence using a two-photon optical fiber probe. Biophys. J. 86 (6), 3959–3965.
Thomas, T.P., Patri, A.K., Myc, A., Myaing, M.T., Ye, J.Y., Norris, T.B., et al., 2004b. In vitro targeting of synthesized antibody-conjugated dendrimer nanoparticles. Biomacromolecules 5 (6), 2269–2274.
Timme, T.L., Hall, S.J., Barrios, R., Woo, S.L., Aguilar-Cordova, E., Thompson, T.C., 1998. Local inflammatory response and vector spread after direct intraprostatic injection of a recombinant adenovirus containing the herpes simplex virus thymidine kinase gene and ganciclovir therapy in mice. Cancer Gene Ther. 5 (2), 74–82.
Tomalia, D.A., Baker, H., Dewald, J., Hall, M., Kallos, G., Martin, S., et al., 1985. A new class of polymers: starburst-dendritic macromolecules. Polym. J. 17 (1), 117.
Turnbull, W.B., Stoddart, J.F., 2002. Design and synthesis of glycodendrimers. J. Biotechnol. 90 (3–4), 231–255.
Uppuluri, S., Swanson, D.R., Piehler, L.T., Li, J., Hagnauer, G.L., Tomalia, D.A., 2000. Core–shell tecto (dendrimers): I. Synthesis and characterization of saturated shell models. Adv. Mater. 12 (11), 796–800.
Vannucci, L., Fiserova, A., Sadalapure, K., Lindhorst, T.K., Kuldova, M., Rossmann, P., et al., 2003. Effects of *N*-acetyl-glucosamine-coated glycodendrimers as biological modulators in the B16F10 melanoma model in vivo. Int. J. Oncol. 23 (2), 285–296.

Vembu, S., Pazhamalai, S., Gopalakrishnan, M., 2015. Potential antibacterial activity of triazine dendrimer: synthesis and controllable drug release properties. Bioorg. Med. Chem. 23 (15), 4561–4566.
Wang, H., Zheng, L., Guo, R., Peng, C., Shen, M., Shi, X., et al., 2012. Dendrimer-entrapped gold nanoparticles as potential CT contrast agents for blood pool imaging. Nanoscale Res. Lett. 7 (1), 190.
Wang, C., Li, Z., Liu, B., Liao, Q., Bao, C., Fu, H., et al., 2013. Dendrimer modified SWCNTs for high efficient delivery and intracellular imaging of survivin siRNA. Nano Biomed. Eng. 5 (3).
Wiener, E.C., Konda, S., Shadron, A., Brechbiel, M., Gansow, O., 1997. Targeting dendrimer-chelates to tumors and tumor cells expressing the high-affinity folate receptor. Invest. Radiol. 32 (12), 748–754.
Wiwattanapatapee, R., Carreno-Gomez, B., Malik, N., Duncan, R., 2000. Anionic PAMAM dendrimers rapidly cross adult rat intestine in vitro: a potential oral delivery system? Pharm. Res. 17 (8), 991–998.
Wolinsky, J.B., Grinstaff, M.W., 2008. Therapeutic and diagnostic applications of dendrimers for cancer treatment. Adv. Drug Deliv. Rev. 60 (9), 1037–1055.
Yamaguchi, H., Harada, A., 2003. Antibody dendrimers. Top. Curr. Chem. 228, 237–258.
Yamashita, S., Katsumi, H., Hibino, N., Isobe, Y., Yagi, Y., Kusamori, K., et al., 2017. Development of PEGylated carboxylic acid-modified polyamidoamine dendrimers as bone-targeting carriers for the treatment of bone diseases. J. Control. Release 262, 10–17.
Yang, H., Kao, W.J., 2006. Dendrimers for pharmaceutical and biomedical applications. J. Biomater. Sci. Polym. Ed. 17 (1–2), 3–19.
Yang, H., Kao, W.J., 2007. Synthesis and characterization of nanoscale dendritic RGD clusters for potential applications in tissue engineering and drug delivery. Int. J. Nanomed. 2 (1), 89–99.
Yang, H., Lopina, S.T., DiPersio, L.P., Schmidt, S.P., 2008. Stealth dendrimers for drug delivery: correlation between PEGylation, cytocompatibility, and drug payload. J. Mater. Sci. Mater. Med. 19 (5), 1991–1997.
Zamore, P.D., Tuschl, T., Sharp, P.A., Bartel, D.P., 2000. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 101 (1), 25–33.
Zhao, M., Meng, L., Ma, L., Wu, G., Xie, F., Ma, L., et al., 2017. Stepwise growth of melamine-based dendrimers onto carbon fibers and the effects on interfacial properties of epoxy composites. Technology 138, 144–150.
Zhou, Z., Lu, Z.-R., 2013. Gadolinium-based contrast agents for magnetic resonance cancer imaging. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol 5–1–18.
Zinselmeyer, B.H., Mackay, S.P., Schatzlein, A.G., Uchegbu, I.F., 2002. The lower-generation polypropylenimine dendrimers are effective gene-transfer agents. Pharm. Res. 19 (7), 960–967.

Further reading

Boas, U., Heegaard, P.M., 2004. Dendrimers in drug research. Chem. Soc. Rev. 33 (1), 43–63.
Cagin, T., Wang, G., Martin, R., Breen, N., Goddard, W., 2000. Molecular modeling of dendrimers for nanoscale applications. Nanotechnology 11, 77–84.
McNerny, D.Q., Leroueil, P.R., Baker, J.R., 2010. Understanding specific and nonspecific toxicities: a requirement for the development of dendrimer-based pharmaceuticals. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2 (3), 249–259.