DENDRIMERS

1. Introduction

Prior to 1984, only three synthetic polymer architectures were known, namely: (*1*) linear, (*2*) cross-linked, and (*3*) branched-type configurations. The "dendritic state" is a new, fourth class of polymer architecture, consisting of five subclasses: (*1*) random hyperbranched polymers, (*2*) dendrigrafts, (*3*) dendrons, (*4*) dendrimers, and (*5*) tecto(dendrimers) or megamers (Fig. 1) (1,2). The monodisperse nature of dendrimers makes them important building blocks for applications in nanomedicine and nanotechnology. These structures differ from traditional polymers in critical nanoscale parameters, such as size, shape, and presentation of chemical functionality. Their architecture (ie, their cores, interiors, and surfaces) can be precisely controlled. The core determines size, shape, directionality, and multiplicity of surface functionality. Within the interior, the length and amplification of branches define the volume and type of containment space enclosed by the terminal groups, offering a variety of possible guest–host interactions. Finally, the surface consists of reactive or passive terminal groups. These may serve as polyvalent nanoscaffolding, upon which new generations of dendrimers can be covalently attached for further growth. Alternatively, the surface groups may function as control gates for the entry and departure of guest molecules from the interior (3). The core, interior, and surface determine all the properties of dendrimers. With the exception of biological polymers, or perhaps fullerenes, no other covalent structure offers such "bottom-up" control. Precise dendritic synthesis strategies (ie, divergent–convergent) have been reported, leading to >100 dendrimer compositional families possessing >1000 different surface/interior chemistries (4,5).

In nanomedicine, the development of well-defined molecular level nanostructures is of eminent importance for applications, such as drug delivery, gene transfection, and diagnostic imaging. For example, constructs utilized as carriers in drug delivery generally should be in the nanometer range and uniform in size to enhance their ability to cross cell membranes and reduce the risk of undesired clearance from the body through liver and spleen. Currently, two traditional routes to prototypes that will meet some of these requirements have been widely investigated. The first route takes advantage of a biological phenomenon, ie, the ability of amphiphilic molecules (molecules consisting of a hydrophilic and hydrophobic moiety) to self-assemble in water above a system-specific critical concentration (ie, CMC) to form structures, such as micelles and liposomes (6,7). The second route relies on engineering the production of well-defined particles through processing protocols, such as shearing or homogenization of oil-in-water (o/w) emulsions or w/o/w double emulsions, extrusion of polymer strands or viscous gels through nozzles of defined size, and layer-by-layer (LbL) deposition of polyelectrolytes and other polymeric molecules around colloidal cores. Size, degree of polydispersity, and stability of these structures vary depending on the systems that are being used in these applications (8).

In nanotechnology applications, growth and development will be largely dependent on successfully identifying appropriate quantized building blocks. The challenge is to develop critical structure-controlled methodologies to produce

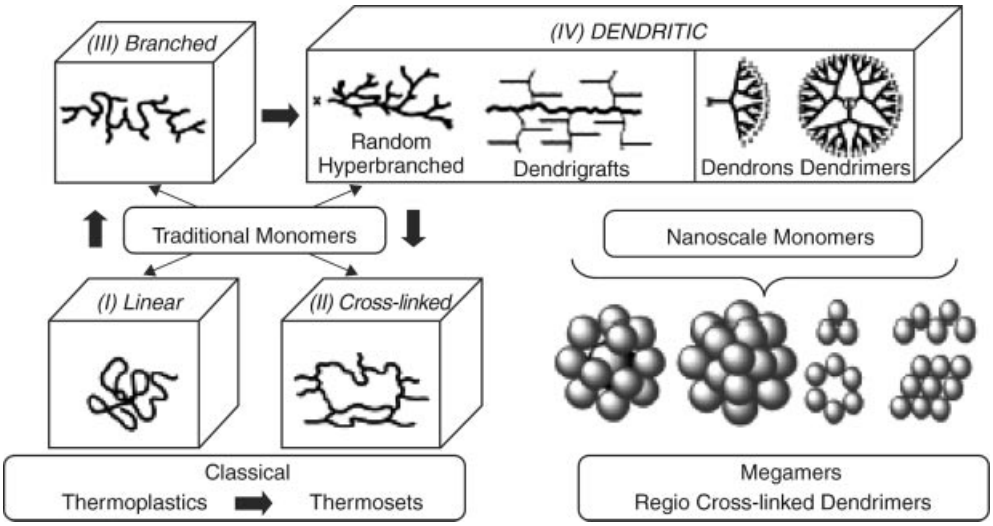


Fig. 1. Schematic representation of classical and dendritic polymer structures. (Reproduced with permission from Ref. 3. Copyright © Elsevier Ltd. 2005.)

appropriate nanoscale modules that will allow cost-effective synthesis and controlled assembly of more complex nanostructures in a very routine manner. Such structures will be macromolecular, require the controlled assembly of as many as 10^3–10^9 atoms, and possess molecular weights ranging from 10^4 to 10^{10} Da.

2. Dendrimer Synthesis

The three traditional macromolecular architectural classes are widely recognized to generate rather polydisperse products of different molecular weights. In contrast, the synthesis of dendrimers offers the opportunity to generate monodisperse macromolecular architectures similar to those observed in biological systems. Since 1979, two major strategies have evolved for dendrimer synthesis. The first, introduced by Tomalia and co-workers, was the *divergent method* in which growth of a dendron originates from a core site (Fig. 2). This approach involves assembling monomeric modules in a radial, branch-upon-branch motif according to certain dendritic rules and principles (9). The second method, pioneered by Fréchet and co-workers, follows a *convergent growth process* (10). It proceeds from what will become the dendrimer surface inward to a reactive focal point, leading to the formation of a single reactive dendron. To obtain a dendrimer structure, several dendrons are reacted with a multifunctional core to yield such a product. Using these two key synthetic strategies, >100 compositionally different dendrimer families have been synthesized and >1000 differentiated chemical surface modifications have been reported (11–16). Most divergent dendrimer syntheses require excess monomer loading and lengthy chromatographic separations, particularly at higher generations. On the other

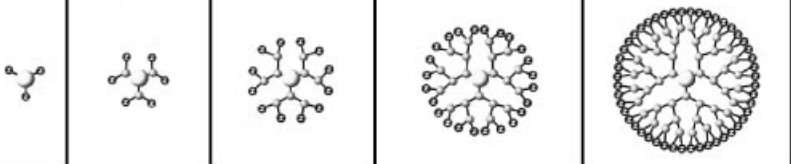
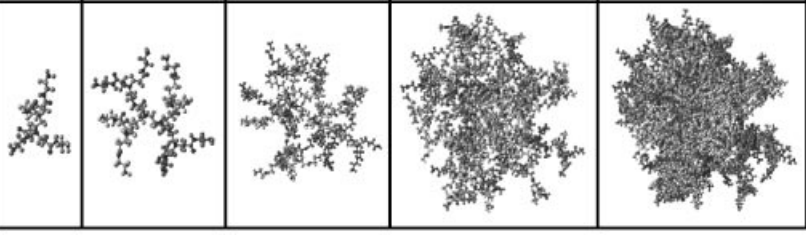
Generation	G0	G1	G2	G3	G4
# of Surface Groups	3	6	12	24	48
Diameter (nm)	1.4	1.9	2.6	3.6	4.4
2D Graphical Representation					
3D Chemical Structure View					

Fig. 2. Schematic representation of radial divergent growth of dendrimers starting from a core molecule (generation G0) to a dendrimer generation G4. The two-dimensional (2D) graphs clearly show the core, interior with branches and the surface functionalities. The three-dimensional (3D) graphs show the overall shape and increasingly dense packing of surface groups. (Copyright © Dendritic NanoTechnologies, Inc. 2006.)

hand, convergent synthesis strategies are generally limited to the construction of only lower generation dendrimers due to nanoscale steric issues that are encountered when attaching the dendrons to the molecular level core. These issues are reviewed and considered elsewhere (5).

Simplifying the synthetic preparation of dendrimers thus has been a major challenge and an obstacle for the commercial utilization of these unique structures in industrial areas that require large quantities of inexpensive materials. Very recently, two new breakthrough approaches in dendrimer synthesis have been reported. The first strategy approach, coined "lego" chemistry, utilizes highly functionalized cores and branched monomers to create phosphorus dendrimers. Several variations of the general synthetic scheme have been developed, allowing multiplication of the number of terminal surface groups from 48 to 250 in one step, eg. These dendrimers require just one step per generation performed in a minimum volume of solvent, allow easy purification (ie, simple washings), and produce environmentally benign by-products, such as water and nitrogen (17–19). The second approach is based on "click" chemistry ie, the near-perfect reliability of the Cu(I)-catalyzed synthesis of 1,2,3-triazoles from azides and alkynes to produce dendrimers with various surface groups in high purity and excellent yield. All generation 2 and some generation 3 dendrimers were isolated directly as pure solids without chromatographic separations, and the only major by-product formed in the reaction is sodium chloride (20–24).

3. Dendrimers as Nanoscale Containers

The core and interior shells of dendrimers represent well-defined nanoenvironments which, in the case of higher generation dendrimers, are protected from the outside by the dendrimer surface (nanoscale containers). These three domains can be tailored for a specific purpose, ie, to function as a dendritic sensor, drug carrier, or as a drug. The high density of exo-presented surface functionalities makes the dendritic surface well suited as a nanoscaffold, where the close proximity of functional groups is important (polyvalency), or for receptor-mediated targeting purposes. On the other hand, the interior is well suited for host–guest interaction and encapsulation of guest molecules. Transmission electron micrographs (TEM) of sodium carboxylate poly(amidoamine) (PAMAM) dendrimers revealed topologies reminiscent of regular classical micelles. Supporting this observation, Turro and co-workers designed a hydrophobic 12-carbon atom alkylene chain into the core of a homologous series of PAMAM dendrimers (G2, 3, and 4) to mimic the hydrophobic and hydrophilic core-shell topology of a regular micelle. The hosting properties of this series toward a hydrophobic guest molecule were then compared with a PAMAM dendrimer series possessing nonhydrophobic cores (25).

The micelle-mimetic behavior of dendrimers was also observed in recent molecular dynamic studies. Depending on the conditions of the bulk solution, ie, its polarity, ionic strength, and pH, dendrimers adopt conformations of different shape and density. For example, poly(propylene imine) (PPI) and PAMAM dendrimers with primary amines as surface groups exhibit extended conformations upon lowering the pH due to electrostatic repulsion between protonated tertiary amines in the interior, as well as between surface primary amines, thus forcing the dendritic branches apart. At pH > 9, backfolding occurs as a consequence of hydrogen bonding between interior protonated tertiary amines and surface primary amines, resulting in a denser interior (Fig. 3) (26). Of course, these pH-related conformational changes are dependent on the charge of the

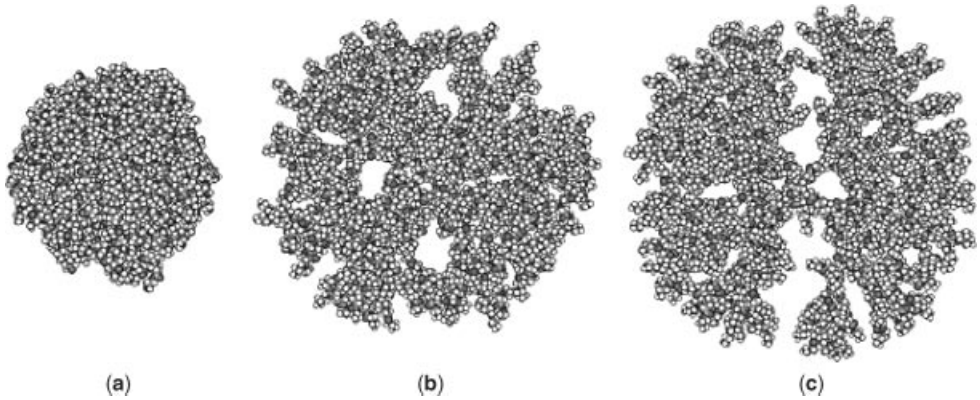


Fig. 3. Final configuration of a G6 PAMAM dendrimer after 100-ps MD simulations (**a**) at high pH (no amine is protonated), (**b**) at neutral pH (primary amines are protonated), and (**c**) at low pH (all amines are protonated). (Reproduced with permission from Ref. 26.)

respective functional groups. For PPI dendrimers having surface carboxylic groups instead of amines, small angle neutron scattering (SANS) and nuclear magnetic resonance (nmr) measurements show that these dendrimers possess the most extended conformations at pH 4 and 11. This observation has been related to electrostatic repulsion between interior cationic protonated tertiary amines at low pH, and electrostatic repulsion between surface anionic deprotonated carboxylates at high pH. Either positive or negative repulsions are forcing the dendritic branches apart. At pH 6, carboxylate-terminated PPI dendrimers exhibit no net charge, resulting in a tighter conformation controlled by intramolecular hydrogen bonds between terminal groups and groups in the core (27). In addition to the pH of the bulk solution, the solvent polarity will effect the dendrimer conformation in a similar way as observed for micelles. Recent nmr studies performed on polar PPI dendrimers indicate that apolar solvents will favor intramolecular interactions (ie, hydrogen bonds), resulting in backfolding of the dendrimer arms into the interior, whereas more polar solvents will compete for and thus weaken these intramolecular interactions in favor of solvated arms, allowing a more extended conformation of the dendrimers (28). These solvent-dependent conformational changes will reverse in the case of less polar dendrimers (ie, dendrimers containing aryl groups or other hydrophobic moieties as building elements), thus forcing these dendrimers to behave as inverse micelles (29). A critical property difference relative to micelles is the increased density of surface groups with higher generations. At some generational level, the surface groups will reach the so-called "de Gennes dense packing" limit and seal the interior from the bulk solution. This limit depends on the strength of intramolecular interactions between adjacent surface groups, and therefore, on the condition of the bulk solution (ie, pH, polarity, temperature). This feature, coined by Meijer and co-workers as "dendritic box" (30) can be utilized to tailor the encapsulation and release properties of dendrimers, eg, in drug delivery applications. The maximum amount of entrapped guest molecules is directly proportional to the shape and size of the guest molecules, as well as to the amount, shape, and size of the available internal dendrimer cavities (30–32).

Specific binding of guest molecules to the dendrimer core can affect the loading capacity by enhancing specific interactions between the core and guest, ie, hydrophobic and polar interactions. Dendrimers specifically tailored to bind hydrophobic guests to the core have been created by Diederich and co-workers and coined "dendrophanes". These water-soluble dendrophanes are built around a cyclophane core, and can bind aromatic compounds, presumably via p–p interactions. Dendrophanes were shown to be excellent carriers of steroids (33,34). The same group synthesized dendrimers tailored to bind more polar bioactive compounds to the core, coined "dendroclefts" (35). An alternative approach to creating dendritic hosts with highly selective guest recognition utilized the principle of "molecular imprinting" (36). A dendrimer consisting of a porphyrin core and a surface containing terminal double bonds was polymerized into a polydendritic network. Subsequently, the base-labile ester bonds between cores and dendritic wedges were cleaved, releasing the porphyrin core from the dendritic polymer. This polymer was capable of selectively binding porphyrins with association constants of $1.4 \times 10^5\ M^{-1}$. Very recently, an impressive approach has been presented, using tandem mass spectrometry, ie, the combination of

electrospray ionization (ESI) and collision-induced dissociation (CID) mass spectrometers connected in series, to investigate the dynamic behavior of host–guest dendrimer complexes (37). This approach offers the potential to provide better insights into these constructs.

4. Medical Applications

The use of dendrimers as drug delivery devices and gene transfection agents has been thoroughly reviewed very recently (38–42). Therefore, only a few highlights are summarized together with additional recent studies.

4.1. Dendrimers in Gene Transfection. Dendrimers and dendrons are very actively under investigation for the delivery of DNA. Numerous reports have been published describing the use of amino-terminated PAMAM or PPI dendrimers as nonviral gene-transfer agents, enhancing the transfection of DNA by endocytosis and, ultimately, into the cell nucleus (41,43–50). The influence of dendrimer generation (44) and core structure (45,46) on gene transfection efficiency has been studied, as well as the nature of the dendrimer–DNA complexes (47). Note that dendrimers of high structural flexibility and partially degraded high generation dendrimers (ie, hyperbranched architectures) appear to be better suited for certain gene delivery applications than intact high generation symmetrical dendrimers. Perhaps this is due to their enhanced flexibility, which allows the formation of more compact complexes with DNA (51,52). Furthermore, it has been found that maximum transfection efficiency is obtained with a net positive charge on the complexes (ie, an excess of primary amines over DNA phosphates). To gain a better understanding of the rules that govern dendrimer-based gene delivery, a series of amphiphilic dendrimers based on the rigid diphenylethyne core was synthesized and their activity as transfection agents described. These dendrimers featured a variety of geometries and substitution patterns, all of which showed high transfection activity, but also brought a couple of surprises. A sharp maximum in the structure–activity relationship was observed based on the hydrophobic component of the dendrimer. The hydrophobic parameters influenced the DNA binding and transport more strongly than anticipated, exhibiting lower toxicity and an unusual serum effect. In contrast to classical cationic dendrimers, these dendrimers did not show a minimum size limitation for transfection (53). Conjugation of α-, β-, and γ-cyclodextrins to the surface of PAMAM dendrimers improved the transfection efficiency, especially using α-cyclodextrin in a 2.4:1 ratio covalently bound to the G3 dendrimer surface. The measured transfection efficiency was ∼100-times higher for the dendrimer conjugate than for the dendrimer alone or of a physical mixture between dendrimer and α-cyclodextrin (54,55).

The physical mixing of linear anionic oligonucleotides (ie, sequence lengths between 6 and 55 bases), as well as hairpin conformations with plasmid DNA prior to the addition of either a commercially available PAMAM dendrimer-based product (SuperFect) or generations 3-5 phosphorus dendrimers was also examined in an attempt to improve the transfection efficiency. While the efficiency increased with the length of the oligonucleotide up to the 36-mer, the conformation of the oligomer was of less importance. It was concluded that the

presence of these charged macromolecules reduced the packing density of the dendrimer–DNA complex, and its enhanced flexibility resulted in improved transfection (56). In another study, dendritic amidoamine side chains of different generations were covalently attached to the polysaccharide chitosan in an attempt to combine the biological activity of chitosan with the delivery benefits found for dendrimers (57). Amphiphilic PAMAM dendrons, generations 1–4, were synthesized utilizing di-*n*-dodecylamine as the core. It was anticipated that the hydrophobic component would mimic the membrane transfection ability of natural phospholipids and enhance membrane penetration. These constructs formed facile complexes with DNA and, in case of the generation 2–4 dendrons, were able to cross cell membranes and efficiently deliver DNA (58). In an extension of this concept, amphiphilic lysine-based dendrons were synthesized, which self-assembled in water to form dendron-based liposomes (dendrisomes) as potential transfection agents (59). Very recently, dendrons with spermine surface (60) and PAMAM dendron–poly(L-lysine) copolymers have been produced (61) and enhanced transfection efficiency has been reported.

Several recent studies combined dendrimers or dendritic structures with amino acids and peptides to improve the delivery ability of amino acid based devices or to create more biocompatible molecules. Monodisperse, dendritic poly(L-lysine)s at several generational levels were prepared in order to compare the gene transfection properties against linear and branched poly(lysine) architectures. Poly(lysine) dendrimers possessing 64 and 128 surface amino groups exhibited efficient gene transfection properties in several cultivated cell lines without significant cytotoxicity (62). Polycationic β-alanine-based dendrimers, generations 2–4, were synthesized and characterized. These molecules are expected to be efficient gene transfection agents due to their structural resemblance to natural biomolecules (63). Surface functionalization of polyphenylene dendrimers with up to 16 lysine residues or short peptide sequences was carried out in order to utilize these compounds as models to study DNA complexation and condensation and as building blocks for novel supramolecular architectures (64). Using L-arginine instead of L-lysine on the surface of PAMAM dendrimers resulted in enhanced transfection efficiency in primary rat vascular smooth muscle cells (65). The more hydrophobic amino acid phenylalanine attached to the surface of PAMAM dendrimers gave enhanced transfection efficiency in African green monkey kidney cells with an increasing number of phenylalanine residues, until solubility problems arose. However, attachment of L-leucine residues did not improve the transfection efficiency (66).

A dendrimer-based transfection agent (SuperFect) from Qiagen Inc. is on the market. SuperFect reagent consists of activated-dendrimer molecules with a defined spherical architecture. Branches radiate from a central core and terminate at charged amino groups that can then interact with negatively charged phosphate groups of nucleic acids. SuperFect reagent assembles DNA into compact structures that bind to the cell surface and are taken into the cell by nonspecific endocytosis. The reagent buffers the pH of the endosome, leading to pH inhibition of endosomal nucleases, which ensures stability of SuperFect–DNA complexes (67).

4.2. Dendrimers in Drug Delivery. In addition to DNA, dendrimers have been successfully utilized to carry a variety of small molecule pharmaceuticals.

Encapsulation of the well-known anticancer drug cisplatin within PAMAM dendrimers gave conjugates that exhibited slower release, higher accumulation in solid tumors, and lower toxicity compared to free cisplatin (68–70). Similarly, the encapsulation of silver salts within PAMAM dendrimers produced conjugates exhibiting slow silver release rates and antimicrobial activity against various Gram positive bacteria (71). The PAMAM dendrimers carrying a biocompatible hydroxyl-surface were able to encapsulate small acidic molecules, such as benzoic acid and 2,6-dibromo-4-nitrophenol, but did not form a complex with the nonacidic drug tioconazole. Presumably, the guest molecules were retained within the dendritic branching clefts by hydrogen bonding with interior protonated amide groups (31).

In another study, two polyester-based dendrimers generation 4 were synthesized, one carrying a hydroxyl surface, the other a tri(ethylene glycol) monomethyl ether surface. These dendrimers were compared to a three-arm poly(ethylene oxide) star polymer, carrying generation 2 polyester dendrons at the surface. The star polymer gave the most promising results regarding low cytotoxicity and long systemic circulatory half-life (72 h). Therefore, the anticancer drug doxorubicin was covalently bound to this carrier via an acid-labile hydrazone linkage. The cytotoxicity of doxorubicin was significantly reduced (80–98%), and the drug was successfully taken up by several cancer cell lines (72). The anticancer drugs adriamycin and methotrexate were encapsulated into generations 3 and 4 PAMAM dendrimers, carrying poly(ethylene glycol) monomethyl ether chains (PEG_{550} and PEG_{2000}), attached via urethane bonds, on their surfaces. The highest encapsulation efficiency within average 6.5 adriamycin molecules and 26 methotrexate molecules per dendrimer was found for the G4 PAMAM terminated with PEG_{2000} chains. Drug release from this dendrimer was slow at low ionic strength, but fast in isotonic solution (73). A similar behavior was observed for the anticancer drug 5-fluorouracil, encapsulated into G4 PAMAM dendrimers capped with PEG_{5000} surface chains. Reasonable drug loading and sustained release rates have been reported (74). Methotrexate conjugated to the surface of G5 PAMAM dendrimers, which also carried the targeting ligand folic acid, were successfully tested for cell uptake *in vitro* using KB cancer cells (75), as well as *in vivo* using immunodeficient mice bearing human KB tumors that overexpress the folic acid receptor (76). The conjugates were injected intravenously (i.v.) and concentrated in the tumor and liver tissue >4 days after administration. Confocal microscopy confirmed internalization of the drug conjugates into tumor cells. Targeting methotrexate increased its antitumor activity and markedly decreased its toxicity, allowing therapeutic responses not possible with the free drug. Following the same approach, the drug paclitaxel was conjugated to the surface of G5 PAMAM dendrimers and tested *in vitro* for targeted delivery (77). Polyglycerol dendrimers mixed with paclitaxel notably enhanced the aqueous solubility of the drug, overcoming a major setback for its bioavailability (78).

The nonsteroidal antiinflammatory drug (NSAID) ibuprofen was used as a model compound to study its complexation and encapsulation into generations 3 and 4 PAMAM dendrimers and a hyperbranched polyester having ~ 128 surface OH groups. It was found that up to 78 ibuprofen molecules were complexed by the PAMAM dendrimers through electrostatic interactions between the dendrimer

amines and the carboxyl group of the drug. In contrast, up to 24 drug molecules were encapsulated into the hyperbranched polyol. The drug was successfully transported into lung epithelial carcinoma cells by the dendrimers (79). In a similar way, the NSAID ketoprofen, naproxen, and diflunisal were complexed with PAMAM dendrimers, and enhanced solubility in water has been reported (80). The effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility, and therefore, bioavailability of nifedipine has been studied. The solubility enhancement of nifedipine was higher in the presence of ester-terminated dendrimers than their amino-terminated analogues possessing the same number of surface groups. Not unexpected, the nifedipine solubility increased with the size of the dendrimers (81). Furthermore, recent work has shown that PAMAM dendrimers enhanced the bioavailability of indomethacin in transdermal delivery applications (82) (Fig. 4). Branched poly(L-glutamic acid) chains were centered around PAMAM dendrimers generations 2 and 3 to create new biodegradable polymers with improved biodistribution and targeting ability. These constructs were surface-terminated with (*1*) PEG chains to enhance their biocompatibility and (*2*) folic acid ligands to introduce cell-specific targeting (83). Star-shaped polylactide (PLA) was synthesized by bulk polymerization with a G1 PAMAM dendrimer as initiator. Unlike linear PLA of similar molecular weight, the branched construct had higher hydrophilicity and faster degradation rate with significantly accelerated release of water-soluble bovine serum albumin (BSA) as the model drug. These constructs are assumed to have potential in the delivery of hydrophilic drugs in tissue engineering, including growth factor and antibodies to induce tissue regeneration (84).

Two recent studies employed dendrimers as building blocks in the construction of new drug delivery devices. The first study extended the dendrimer–PEO motif by designing "bow-tie" structures, ie, covalently connecting two polyester dendrons, where one dendron provides multiple functional handles for the attachment of drug molecules, while the other dendron is used for the attachment of solubilizing poly(ethylene oxide) chains. By varying the generations of

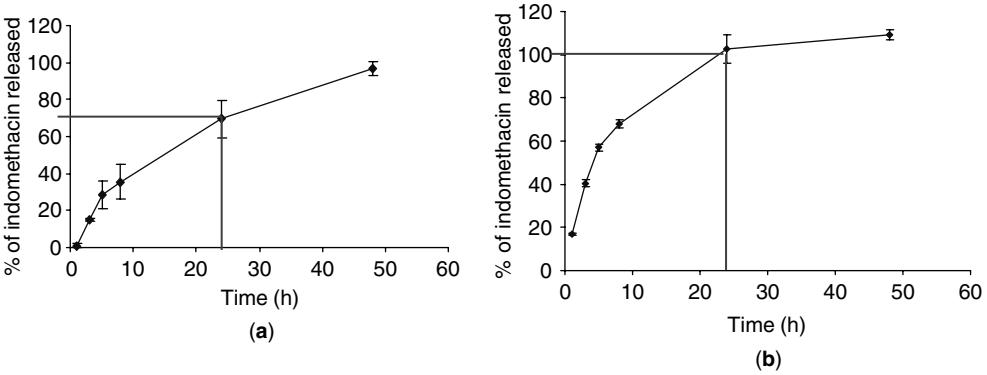


Fig. 4. Indomethacin release from PAMAM dendrimers. (**a**) G4 PAMAM with PEG surface; and (**b**) G3.5 PAMAM with carboxylate surface. The release profiles indicate a size and surface effect. (Copyright © Dendritic NanoTechnologies, Inc. 2006.)

dendrons and the mass of the PEO chains, molecular weight, architecture, and drug loading capacity could be readily controlled. A library of eight carriers with molecular weights between 20 and 160 kDa was synthesized and characterized (85). The other study employed generation 4 PAMAM dendrimers to construct dendrimer–poly(styrenesulfonate) (PSS) microcapsules following LbL deposition of both constituents around a removable melamine formaldehyde colloidal core. These PAMAM–PSS capsules are expected to allow the selective encapsulation of drug into the capsule core and into the dendrimers, which are localized within the shell of the capsule, thus providing a dual release system of either two different drugs (ie, drug cocktail) or of one drug released following two different time protocols (ie, fast and sustained release). Doxorubicin hydrochloride has been used as a model compound for preliminary encapsulation and release studies (86).

4.3. Dendrimers as Imaging Agents. Dendrimers are under investigation as imaging agents for magnetic resonance imaging (MRI), *in vivo* oxygen imaging, and computed tomography (CT) imaging. In MRI, chelates of paramagnetic metal ions, such as gadolinium(III) (ie, Gd(III)–DOTA and Gd(III)–DTPA) increase the relaxation rate of surrounding water protons (87,88). The Gd(III)–DTPA conjugate is known commercially as Magnevist (Schering AG) and is a widely used MRI contrast agent. However, shortcomings of these low molecular weight contrast agents are short circulation times within the body and inefficient discrimination between diseased and normal tissues. Subsequently, macromolecular Gd(III) complexes have been developed by conjugating Gd(III) chelates to biomedical polymers, including poly(amino acids), polysaccharides, and proteins to improve image contrast enhancement. These macromolecular agents have demonstrated superior contrast enhancement for blood pool imaging and cancer imaging in animal models. Unfortunately, the clinical application of macromolecular agents in general is limited by their slow excretion rate and the resulting accumulation within the body. In addition, the long residence time of MRI agents enhances the risk of potential toxicity by Gd(III) ions released during the metabolism of these agents (89–91). Dendrimer-based MRI contrast agents have been studied extensively *in vivo* during the last decade. For example, dendrimer-based Gd(III) chelates consisting of generations 2 and 6 PAMAM dendrimers with 12 and 192 terminal surface amines conjugated to the chelating ligand 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid have been synthesized and used *in vivo* with rabbits. These contrast agents exhibited excellent MRI images of blood vessels upon i.v. injection. The blood circulation times were sufficiently long, with $>$ 100 minutes for large dendrimer conjugates, such as the G6 PAMAM–Gd(III)–DTPA. The results were confirmed by studies employing generations 7, 9, and 10 PAMAM dendrimer–Gd(III) chelates containing up to 1860 Gd(III) ions per dendrimer. In this study, the total molecular relaxivity increased strongly with the molecular weight, although relaxivity saturation was observed (92). Clearance times of dendrimer-based MRI agents and the potential risk of Gd(III) release were the subject of another study. Six small dendrimer-based agents were synthesized, based on either PAMAM or diaminobutane (DAB) dendrimers, and their pharmacokinetics, whole-body retention, and dynamic MRI were evaluated. The DAB-based agents cleared more rapidly from the body than PAMAM dendrimer-based agents with the same number of surface groups. As a result of this

study, one generation 2 PAMAM and two generations 2 and 3 DAB dendrimers were identified as contrast agents with potential for use in clinical applications (93). Most interestingly, in a recent study it was found that the molecular size of a dendrimer-based MRI agent altered the route of excretion. Contrast agents with molecular weight < 60 kDa were excreted through the kidney, resulting in these agents being potentially suitable as functional renal contrast agents. Hydrophilic and larger sized contrast agents were found better suited for use as blood pool contrast agents. Hydrophobic variants formed with DAB dendrimer cores created liver contrast agents. Larger hydrophilic agents were useful for lymphatic imaging. Finally, contrast agents conjugated to either monoclonal antibodies or avidin were able to function as tumor-specific contrasts (94).

The potential of these dendrimer-based MRI agents has been recognized by the pharmaceutical industry and resulted in several commercial developments. For example, the 24-Gd(III)–DTPA cascade polymer and dendritic Gadomer 17 have been introduced (95). Gadomer 17 is suitable for blood-pool imaging, similar to the known linear Gd(III)–DTPA–poly(lysine), but shows a superior elimination rate presumably as a result of the globular nature of the dendrimer derivative. A very recent study describes a convenient methodology for the synthesis of Gd(III)–DTPA-terminated poly(propylene imine) dendrimers with tunable molecular relaxivities as contrast agents (96).

In vivo oxygen imaging is a strategy that offers the potential for diagnosing complications from diabetes and peripheral vascular diseases, as well as the detection of tumors and the design of their therapeutic treatment. The technique is based on the quenching of phosphorescence by oxygen and requires the presence of a chromophor (ie, palladium complexes of tetrabenzoporphyrins), which has strong absorption bands in the near-infrared (ir) range to minimize the interference of natural chromophors present in the blood stream. The Pd complex has to be water soluble and protected from interactions with serum-borne macromolecules, such as albumin. It has been shown that encapsulation of Pd complexes into dendrimers of various sizes can be utilized to tune the oxygen quenching of the phosphorescence. Although the dendritic shell used in these experiments appears to be too permeable to oxygen for optimized imaging purposes, there is some optimism in the viability of these dendrimer–Pd complexes in that the quenching constant has been influenced by a factor 5 (97). However, note that phosphorescent porphyrin–dendrimer complexes for superficial tissue oxygen imaging and measurement are being studied quite extensively (98–102) and have been commercialized. The two most commonly used dendritic oxygen imaging agents are Oxyphor R2 and Oxyphor G2 (103).

Novel water-soluble dendritic nanoparticles, ie, generation 4 PAMAM dendrimers with 3-*N*-[(*N*′,*N*′-dimethylaminoacetyl)amino]-α-ethyl-2,4,6,-triiodo benzene-propanoic acid moieties covalently attached to their surfaces have been applied in CT imaging. The high iodine content (33%) makes them superior agents compared to iodinated small molecules for this technology, which provides a reliable and widely available imaging method with high spatial resolution. Furthermore, the longer circulation times of these macromolecular agents allow for an extended imaging timescale and potential reduced toxicity that would accompany repeated injections of high doses of small iodine molecules (104). In summary, dendrimers and dendrons have an immense potential in

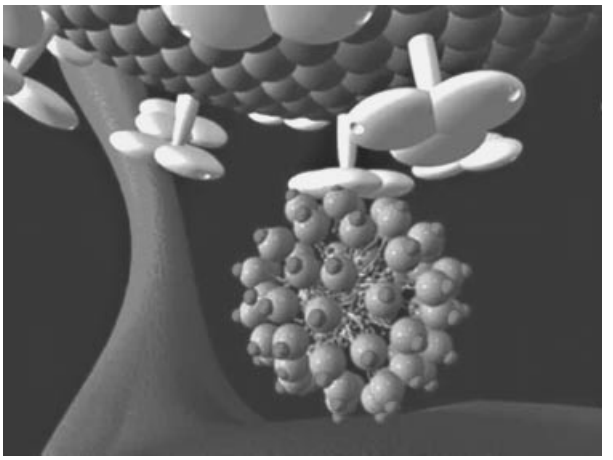


Fig. 5. SPL7013, the polyvalent dendrimer ingredient of Starpharma's topical microbicide, VivaGel, binds to surface proteins on human immunodeficiency virus (HIV), preventing the virus from infecting human T-cells. (http://www.starpharma.com/framemaster.htm, accessed December 9, 2005, with permission from Starpharma Proprietary Limited © 2005.)

the area of molecular imaging with clear leads and many opportunities yet to be explored.

4.4. Dendrimers in Cell Targeting. The surface of dendrimers provides an excellent platform for the attachment of cell-specific ligands (Fig. 5), solubility modifiers, stealth molecules reducing the interaction with macromolecules from the body defense system, and imaging tags. The ability to attach any or all of these molecules in a well-defined and controllable manner onto a robust dendritic surface clearly differentiates dendrimers from other carriers such as micelles, liposomes, emulsion droplets, and engineered particles. One example of cell-specific dendritic carriers is dendrimers modified with folic acid. The membrane-associated high affinity folate receptor (hFR) is a folate-binding protein that is overexpressed on the surface of many cancer cells, and therefore, folate-modified dendrimers would be expected to internalize into these cells preferentially over normal cells via receptor-mediated endocytosis. Folate-dendrimer conjugates have been shown to be well suited for targeted, cancer-specific drug delivery of cytotoxic substances (75,76,105). Very recently, folate–PAMAM dendrimers have been successfully used as carriers of boron isotopes in boron neutron-capture treatment (BNCT) of cancer tumors (106).

In addition to folates, carbohydrates constitute another important class of biological recognition molecules, displaying a wide variety of spatial structures due to their branching and anomericity. To achieve sufficiently high binding affinities between simple mono- and oligosaccharide ligands and cell membrane receptors, these ligands have to be presented to the receptors in a multivalent or cluster fashion (107). The highly functionalized surface of dendrimers provides an excellent platform for such presentations. The design, synthesis, and bio medical use of glycodendrimers as well as their application in diagnostic and for vaccinations have been thoroughly reviewed recently (108–111). For

example, the Thomsen-Friedenreich carbohydrate antigen (T-antigen), β-Gal-(1-3)-α-GalNAc, which has been well documented as an important antigen for the detection and immunotherapy of carcinomas, especially relevant to breast cancer, has been attached to the surface of PAMAM and other dendrimers (112,113). An enhanced binding affinity was observed for all glycodendrimers. These constructs could have potential in blocking the metastatic sites of invasion tumor cells. A series of dendritic β-cyclodextrin derivatives bearing multivalent mannosyl ligands has been prepared and their binding efficiency towards the plant lectin concanavalin A (Con A) and a mammalian mannose-specific cell surface receptor from macrophages has been studied. The effects of glycodendritic architecture on binding efficiency, molecular inclusion, lectin-binding properties, and the consequence of complex formation using the anticancer drug docetaxel on biological recognition were investigated (114). In a similar approach, glycodendrons carrying two to four β-D-galactose moieties on their surface, while the dendron core was connected to a protein-degrading enzyme, were synthesized. These glycodendriproteins are expected to attach to the surface of bacteria, allowing the enzyme to degrade the bacterial adhesin hence rendering the bacteria incapable of attaching to cell surfaces (115). The combined polyvalent interactions of glycodendrons, self-assembled to form nanoparticles, have been utilized to efficiently inhibit polyvalent processes both *in vitro* and *in vivo* (116). Anionic PAMAM dendrimers (G3.5) were conjugated to D(+)-glucosamine and D(+)-glucoseamine 6-sulfate to modulate the immuno and antiangiogenic properties of these particles. It was found that these water-soluble conjugates not only revealed immunomodulatory and antiangiogenic properties, but synergistically prevented scar tissue formation after glaucoma filtration surgery. In a validated and clinically relevant rabbit study, the long-term success rate was increased from 30 to 80% using these dendrimer conjugates (117).

The surfaces of PAMAM dendrimers generations 0–3 were decorated with benzylpenicillin in an attempt to develop a new *in vitro* test to quantify IgE antibodies to specific β-lactam conjugates with the goal of improving the existing methods for diagnosing allergy to this type of antibiotic. The monodispersity of dendrimers is advantageous over conventional peptide carrier conjugates, such as human serum albumin (nonprecise density of haptens in their structure) and poly-L-lysine (mixture of heterogeneous molecular weight peptides). Preliminary radioallergosorbent tests (RAST), using sera from patients allergic to penicillin, have confirmed the usefulness of penicilloylated dendrimers (118).

Monolayers formed by generation 4 PAMAM dendrimers on a gold surface were functionalized with biotin and produced a biomolecular interface that was capable of binding high levels of avidin. Avidin binding as high as 88% coverage of the surface was observed despite conditions that should cause serious steric hindrance. These dendritic monolayers were utilized to study protein–ligand interactions (119).

4.5. Dendrimers as Nanodrugs. Dendrimers have been studied extensively as antitumor, antiviral, and antibacterial, drugs (40). As antitumor drugs, dendrimers are being investigated for their use in photodynamic therapy (PDT). In this application, they are constructed around a light harvesting core, such as a porphyrin. To reduce the toxicity under non-irradiative conditions (dark toxicity), these dendrimers are then encapsulated into micelles, for example

poly (ethylene glycol)-*b*-poly(aspartic acid) micelles. These micelles are stable under physiological conditions (pH 6.2–7.4); however, they disintegrate in the acidic environment (pH ~5.0) of the intracellular endosome (119,120). Alternatively, phthalocyanine–dendrimer conjugates (121) or the photosensitizer 5-aminolevulinic acid, attached to the surface of dendrimers (122), have been studied as agents for PDT. Photosensitive dyes have been incorporated into dendrimers and utilized in PDT devices. For example, uptake, toxicity, and mechanism of photosensitization of the dye pheophorbide A has been compared *in vitro* using human leukemia cells to its complex with DAB dendrimers (123).

Poly(lysine) dendrimers, modified with sulfonated naphthyl groups, have been found useful as antiviral drugs against the *herpes simplex* virus (124). Such a conjugate based on dendritic poly(lysine) scaffolding is VivaGel (Fig. 5), a topical agent currently under development by Starpharma Ltd., Melbourne, Australia, that can potentially prevent/reduce transmission of HIV and other sexually transmitted diseases (STDs). VivaGel (SPL 7013) is being offered as a water-based gel, with the purpose to prevent HIV from binding to cells in the body. The gel differs from physical barriers to STDs, such as condoms, by exhibiting inhibitory activity against HIV and other STDs. In July 2003, following submission of an Investigational New Drug (IND) application, Starpharma gained clearance under US FDA regulations to proceed with a Phase I clinical study to assess the safety of VivaGel in healthy human subjects. This Phase I study, representing the first time a dendrimer pharmaceutical has been tested in humans, compared 36 women who received either various intravaginal doses of VivaGel or a placebo gel daily for 1 week. A thorough review of the data revealed no evidence of irritation or inflammation (125). In earlier studies, it was found that PAMAM dendrimers covalently modified with naphthyl sulfonate residues on the surface, also exhibited antiviral activity against HIV. These dendrimer-based nanodrugs inhibited early stage virus–cell adsorption and later stage viral replication by interfering with reverse transcriptase and/or integrase enzyme activities (126).

The general mode of action of antibacterial dendrimers is to adhere to and damage the anionic bacterial membrane, causing bacterial lysis (40,127). PPI dendrimers with tertiary alkyl ammonium groups attached to their surface have been shown to be potent antibacterial biocides against Gram positive and Gram negative bacteria (128). Poly(lysine) dendrimers with mannosyl surface groups are effective inhibitors of the adhesion of *Escherichia coli* to horse blood cells in a haemagglutination assay, making these structures promising antibacterial agents (129), while chitosan–dendrimer hybrids have been found to be useful as antibacterial agents, carriers in drug delivery systems, and in other biomedical applications. Their behavior has been reviewed very recently (130). Triazine-based antibiotics were loaded into dendrimer beads at high yields. The release of the antibiotic compounds from a single bead was sufficient to give a clear inhibition effect (131). In many cases, dendritic constructs were more potent than analogous systems based on hyperbranched polymers. Dendrimers have been synthesized around a pentanone core, presenting *m*-terphenyl surface groups. These dendrimers were antibacterial against *Salmonella typhi*, *Salmonella pararyphi*, and *Staphylococcus aureus* (132). The antimicrobial peptide, QEKIRVRLSA, has been synthesized in monomeric and dendritic

tetrabranched form. The antibacterial activity against *E. coli* of the dendritic form was superior to the monomeric analogue (133).

4.6. Biocompatibility Studies of Dendrimers. Dendrimers in medical applications have to exhibit low toxicity and be nonimmunogenic in order to be widely useable (Fig. 6). To date, the cytotoxicity of dendrimers has been primarily studied *in vitro*, however, a few *in vivo* studies have been published (40). As observed for other cationic macromolecules, including liposomes and micelles, dendrimers with positively charged surface groups are prone to destabilize cell membranes and cause cell lysis. For example, *in vitro* cytotoxicity IC_{50} measurements (the concentration where 50% of cell lysis is observed) for amino-terminated PAMAM dendrimers revealed significant cytotoxicity on human intestinal adenocarcinoma Caco-2 cells (134,135). Furthermore, the cytotoxicity was found to be generation dependent, with higher generation dendrimers being the most toxic (134,136). A similar generation dependency of amino-terminated PAMAM dendrimers was observed for the hemolytic effect, studied on a solution of rat blood cells (137). However, some recent studies have shown that amino-terminated PAMAM dendrimers exhibit lower toxicity than more flexible amino-functionalized linear polymers, perhaps due to lower adherence of the rigid globular dendrimers to cellular surfaces. The degree of substitution, as well as the type of the amine functionality is important, with primary amines being more toxic than secondary or tertiary amines (136). Amino-terminated PPI dendrimers and PAMAM dendrimers behave similarly with regard to cytotoxicity and hemolytic effects, including the generation-dependent increase of both (52,137).

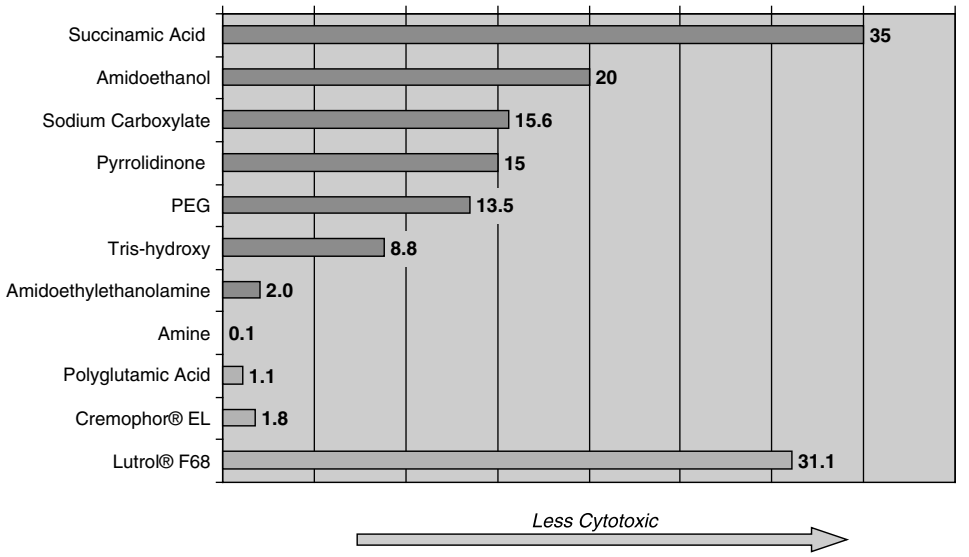


Fig. 6. Cytotoxicity (IC_{50} mg/mL) of PAMAM STARBURST dendrimers with different surface groups, measured against human colon adenocarcinoma, Caco-2, cells. The cytotoxicity of these dendrimers is either similar to or less than commercial excipients, ie, poly(glutamic acid), Cremophore EL and Lutrol F68. (Copyright © Dendritic Nano-Technologies, Inc. 2006.)

Comparative toxicity studies on anionic (carboxylate-terminated) and cationic (amino-terminated) PAMAM dendrimers using Caco-2 cells have shown a significantly lower cytotoxicity of the anionic compounds (134). In fact, lower generation PAMAM dendrimers possessing carboxylate surface groups show neither hematotoxicity nor cytotoxicity at concentrations up to 2 mg/mL (137). The biocompatibility of dendrimers is not solely determined by the surface groups. Dendrimers containing an aromatic polyether core and anionic carboxylate surface groups have shown to be hemolytic on a solution of rat blood cells after 24 h. It is suggested that the aromatic interior of the dendrimer may cause hemolysis through hydrophobic membrane contact (137).

One way to reduce the cytotoxicity of cationic dendrimers may reside in partial surface derivatization with chemically inert functionalities such as PEG or fatty acids. The cytotoxicity toward Caco-2 cells can be reduced significantly (from IC_{50} $\sim$0.13 m*M* to $>$1 m*M*) after such a modification. This observation can be explained by the reduced overall positive charge of these surface-modified dendrimers. A partial derivatization with as few as six lipid chains or four PEG chains on a G4 PAMAM, respectively, was sufficient to lower the cytotoxicity substantially (134).

Only a few systematic studies on the *in vivo* toxicity of dendrimers have been reported so far. Upon injection into mice, doses of 10 mg/kg of PAMAM dendrimers (up to G5), displaying either unmodified or modified amino-terminated surfaces, did not appear to be toxic (124). Hydroxy- or methoxy-terminated dendrimers based on a polyester scaffold have been shown to be of low toxicity both *in vitro* and *in vivo*. At very high concentrations (40 mg/mL), these polyester dendrimers induced some inhibition of cell growth *in vitro* but no increase in cell death was observed. This observation held true for *in vivo* studies in mice, where injection induced neither acute nor long-term toxicity, making these new dendritic motifs very promising candidates for drug delivery devices (72).

5. Technical Applications

Most present dendrimers are still rather expensive and tedious to synthesize. Therefore, there are not many applications outside the medical area, where purity and uniformity are essential and the required volumes comparatively small. However, new approaches in dendrimer synthesis, ie, the "lego" and "click" synthetic routes, will reduce costs and will allow producing dendrimers in large quantities, making them competitive to hyperbranched polymers (138). Following a few technical applications will be highlighted.

5.1. Luminescent Dendrimers. Luminescence can be defined as the emission of light in the ultraviolet (uv), visible (vis), or near-ir range by electronic excited states of atoms or molecules. Luminescence is an important phenomenon for practical applications such as lasers, displays and sensors (139–141). Coupling luminescence with dendrimer chemistry can lead to systems capable of performing very interesting functions, such as (*1*) light harvesting, (*2*) changing the color of light, (*3*) sensing with signal amplification, (*4*) quenching and sensitization processes, and (*5*) shielding effects. Luminescent units can be chemically bound to different regions of a dendritic structure and can also be

noncovalently encapsulated into the voids of a dendrimer or associated at the dendrimer surface (142,143). Luminescent metal complexes, particularly porphyrins, have extensively been used as cores to obtain large dendrimers. Even when the dendrons do not exhibit relevant light absorption and light emission properties, the luminescent properties of the dendrimer may differ from those of the naked core since the appended dendrons can shield the luminescent excited state of the core from quenching by external components such as dioxygen or impurities (144). A recent study compared the shielding ability toward dioxygen quenching of a phosphorescent, Pd-containing porphyrin core by three different types of dendrons, namely, Fréchet-type poly(arylether)s, Newkome-type poly(etheramide)s, and polyglutamates. In aqueous solution, the Fréchet dendrons were most efficient in core shielding. In particular, a strong decline in quenching rate had been observed upon increasing the generation, ie, a 24-times decrease took place between generations 0 and 2, while addition of the next dendritic generation did not result in additional change (145). Dendrimers containing a porphyrin core surrounded by dendrons carrying carbazole molecules as branches have been synthesized and luminescence and redox behavior of these dendrimers as well as the parent dendrons have been studied. All the carbazole dendrons were luminescent at room temperature and at 77 K. The porphyrin-cored dendrimers exhibited luminescence both at room temperature and at 77 K, which was typical of the porphyrin core moieties, regardless of the excitation wavelength. Moreover, excitation spectra fairly overlapped the absorption spectra, showing that all the light absorbed by the peripheral carbazole chromophores was transferred to the porphyrin core. These dendrimers have therefore been regarded as efficient light-harvesting antenna systems, with the porphyrin cores acting as energy traps (146). Multiporphyrin dendrimers have been studied as artificial light-harvesting antennas, mimicking the function of bacterial photosynthetic systems (147).

Luminescent lanthanide reporters emitting in the visible and near-ir domains are in increasing demand due to unique electronic properties, such as long luminescence lifetimes, photostability, and sharp emission bands. These properties make them superior reporters for applications, such as drug discovery, genomic and proteomic screening, medical diagnostics, and biological imaging. A promising strategy to maximize the number of absorbed and emitted photons is the development of reporters that possess more than one luminescent metal ion by synthesizing polymetallic lanthanide complexes. This can be achieved by using large, flexible ligands, such as dendrimers. Attaching sensitizing groups to the end branches of the dendrimer allows for high absorption of the complex, and the presence of multiple coordinating groups within the dendrimer core enables the binding of several luminescent lanthanide ions (148). For example, strong near-ir emission intensity has been found for an Erbium(III) core, generation 2 arylether-functionalized dendrimer complex based on Pt(II)–porphyrin (149).

Well-defined, modular dendrimers enable processing techniques and electronic properties to be tuned independently. Moreover, the dendritic topology can isolate the core chromophore, thus reducing or eliminating strong intermolecular interactions. This behavior was demonstrated by the synthesis of three series of flexible, functionalized dendrimers as red-light emitting materials by

a convergent approach: (*1*) carbazole (CZ) or oxadiazole (OXZ) terminated imide-type dendrimers, (*2*) cascade energy-transferring imide-type dendrimers, and (*3*) CZ-terminated perylene bay-type dendrimers. All dendrimers consisted of the luminescent core perylenebis(dicarboximide), with the specific functional groups of CZ or OXZ at the periphery, and were constructed from flexible Fréchet-type poly(arylether) dendrons (150). A light-blue phosphorescent dendrimer for efficient light-emitting diodes was prepared from biphenyl-based dendrons carrying 2-ethylhexyloxy surface groups connected to a fac-tris [2-(2,4-difluorophenyl)pyridyl]iridium(III) core via a ethane linker to decouple dendrons from the core and preserve the light-blue color of the device (151). A series of luminescent branched Pt(II) alkynyl complexes was synthesized and revealed long-lived emission in both solution and solid-state phases at room temperature. By incorporating different alkynyl ligands into the periphery of these branched complexes, one could readily tune the nature of the lowest energy emissive state and the direction of the excitation energy transfer, again taking full advantage of the control over dendrimer architecture (152).

The use of polymeric light-emitting diodes (LED) for flat panel displays offers many advantages, including large-area fabrication, light weight, flexibility, low operating voltage, and ease of color tuning. Therefore, two novel amphiphilic light-emitting dendrons, consisting of hydrophobic oligo (phenylenevinylene) core branches and hydrophilic oligo(ethylene oxide) terminal chains of generations 1 and 2 have been synthesized and their amphiphilic and fluorescent properties in solution have been studied. These dendrons combine stiff and insoluble conjugated core branches with highly flexible and soluble oligo(ethylene oxide) terminal chains into one macromolecule. Because of the amphiphilic properties arising from the large difference in solubility between the two constituent components, unique concentration-dependent photoluminescence was observed for aqueous solutions of these dendrons. Solution spectra for these amphiphilic fluorescent dendrons displayed red shifts with increasing concentration and/or solvent polarity (153).

The ability to switch the fluorescence of the dye eosin off and on has been demonstrated using dendrimers based on a 1,3,5-trisubstituted benzenoid-type core, containing viologen units in their branches, and terminated with tetraarylmethane derivatives. These highly charged cationic species gave rise to strong host–guest complexes with the dianionic form of the red dye eosin. Upon complexation between the viologen units and eosin, the absorption spectrum of eosin became broader and was slightly displaced toward lower energies, whereas the strong fluorescence of eosin was completely quenched. This host–guest interaction could be destroyed by addition of chloride ions, a procedure that permitted eosin to escape from the dendrimers' interior in a controlled way and to regain its intense fluorescence. When chloride anions were precipitated out of solution by addition of silver cations, eosin molecules reentered the dendrimers' interior and their fluorescence again disappeared (154). These results are of interest for the development of supramolecular systems capable of performing functions related to, eg, sensing applications.

The area of multiphoton applications of dendrimers is becoming increasingly more popular. The dendritic architecture makes dendrimers especially interesting and unique for this type of technology. For example, a novel approach for

the sensitization of singlet oxygen has been developed, which utilizes indirect excitation of the photosensitizer by two-photon-excited fluorescence resonance energy transfer (FRET) from separate chromophores assembled into a dendrimer. This approach effectively enhanced the two-photon excitation efficiency of a known photosensitizer, without the sort of chromophore modifications that could lead to loss of photosensitization and other desirable photophysical properties. Photosensitization of singlet oxygen via excitation wavelengths transmissive to human body tissue (750–1000 nm) could alleviate the depth limitations of photodynamic therapy (155). Wavelength-tunable femtosecond pulses have been used to measure intrinsic (simultaneous) two-photon absorption and three-photon absorption molecular cross-sections in two series of π-conjugated dendrimers built of identical 4,4′-bis(diphenylamino) stilbene (BDPAS) and 4,4′-bis(diphenylamino) distyrylbenzene (BDPADSB) repeat units. Record large two-photon absorption cross sections were obtained for the largest second-generation BDPAS-based dendrimer, as well as zero-generation 4-arm BDPADSB-based dendrimer. In both series, maximum two-photon absorption cross-section increased nonlinearly with the number of π-electrons, whereas for higher generations this dependence turned to linear one (156). Nonlinear absorption properties of dendritic oligofluorenes have been reported as well. The nonlinear absorption has been attributed to a three-photon absorption process involving first a two-photon absorption step followed by an excited state absorption process (157).

5.2. Dendrimer-Stabilized Quantum Dots. The emergence of quantum dot (QD) fluorescence as a nanotechnology application for diagnostics and medicine has taken the industry by storm. Quantum dots are semiconductor nanocrystals that fluoresce when excited by a light source, emitting bright colors that can identify and track properties and processes in various applications. They have significant advantages over traditional fluorophores, particularly in terms of the brightness of the fluorescent signal they can generate, their range of colors and their stability. Quantum dots take advantage of the quantum confinement effect predicted by quantum mechanical theory to fluoresce extremely brightly when excited by a light source such as a laser. By varying the size of the crystals one can cause a rainbow of colors. The QD stay lit for much longer periods of time than conventional dyes, often hours or days. However, these nanoparticles have a strong tendency to aggregate, forming particle clusters with different size-dependent properties.

The tree-like structure of dendrons may be visualized as an ideal shape to stabilize nanocrystals and QD (Fig. 7). Close packing of cone-shaped dendrons around a nanoparticle would create a dense shell at the metal interface, which may be more efficient in passivating the surface than traditional ligand shells formed from flexible hydrocarbon chains or linear polymers. Nanocrystals CdSe/CdS have been dendronized with thiol core functionalized PAMAM dendrons, possessing carboxylic acid surfaces, using the ligand-exchange technique. Results showed that G1 dendrons were not efficient to passivate these QD; however, G2 dendrons appeared to provide adequate surface dense packing to protect the dendronized QD against aggregation for up to 6 months. Some fluorescence quenching (~60%) was observed after ligand exchange was performed but the residual fluorescence intensity was more than adequate to allow the use of these dendronized QD for imaging purposes (158). Nonquenching photoluminescence

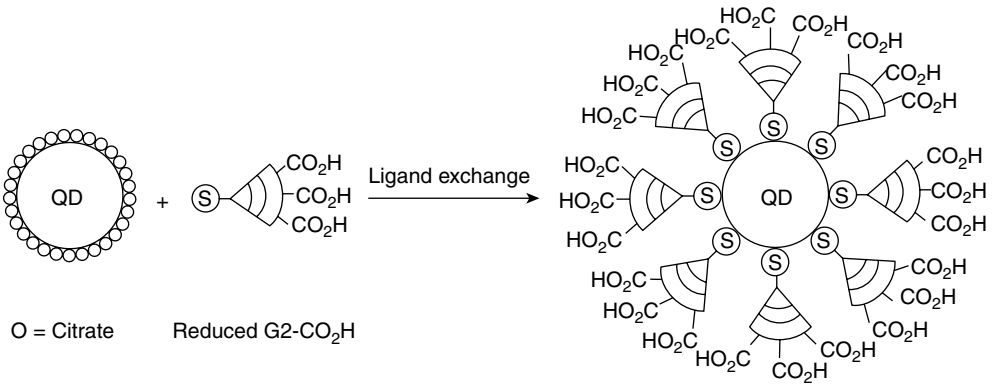


Fig. 7. Illustration of dendron-passivated QD. (Reproduced with permission from Ref. 158. Copyright © Elsevier B.V. 2004.)

properties have been achieved using dendrons featuring a phospine functional group at their focal point instead of a thiol group (159).

Well-defined PAMAM dendrimers have been utilized to encapsulate Au_8 nanoclusters, exhibiting very clean mass spectra, bright blue emission, and improved fluorescence quantum yields (42%) in aqueous solution compared to naked gold nanoclusters. In addition, quantum-confined, water-soluble, high quantum yield Au nanodots with discrete absorption and fluorescence have been prepared from PAMAM encapsulated Au that are highly fluorescent, water soluble, and behave as multielectron artificial atoms with size tunable, discrete electronic transitions between states of well-defined angular momenta (160). Following a similar approach, the self-assembly of π-conjugated polymers, forming a shell around gold nanoparticles, has been utilized to organize these hybrid materials into defined objects and to study their properties as optoelectronic devices (161).

5.3. Dendrimer in Catalysis. Noble metal nanoparticles are attractive for catalyses because of their size effects. However, having atoms with very active surfaces often leads to aggregation of the nanoparticles and decrease in catalytic activity and selectivity. In particular, Pd(0) catalysts are known to aggregate easily and form Pd(0) black, although they catalyze a wide variety of useful reactions in organic syntheses. The PAMAM dendrimers are soft adsorbents that permit passage of substrates and products of the catalytic reactions. Recently, it was reported that dendrimer-encapsulated noble metal clusters exhibited highly catalytic activity, and the dendrimers acted as both templates and porous nanoreactors (162). However, as homogeneous catalysts, difficulties in separation and reuse limit the application of the materials. Immobilization of catalysts on solid supports may result in significant losses of catalytic activities. As a result, much effort has been put into finding new catalytic systems, which effectively combine the advantages of both heterogeneous and homogeneous catalyses. In one approach, dendrimers were grafted onto the surfaces of amine-functionalized SBA-15 channels. These dendrimers were then loaded with Pd(II) ions, which were subsequently reduced to Pd(0). These catalysts showed high catalytic activity for the hydrogenation of allyl alcohol. Importantly,

the hydrogenation rate and selectivity could be controlled by using different generation catalysts. In addition, the catalysts were stable enough to be recycled multiple times and preserved for 1 month under ambient condition, while maintaining the catalytic activities (163).

Catalytic decomposition of carbon tetrachloride at low temperature using supercritical carbon dioxide as the solvent and iron-encapsulating PPI dendrimers as catalyst allowed the synthesis of multiwalled carbon nanotubes (MWNT). This dendritic route is a vast improvement over existing methods. Of the variety of methods used to synthesize carbon nanotubes, catalyzed chemical vapor deposition (CVD) represents the current method of choice. Although CVD offers the benefit of significantly lower deposition temperatures than arc-based techniques, the common temperature range of 600–1000°C precludes the use of temperature-sensitive substrates. Hence, there is much interest in lowering the growth temperature, while maintaining high yields and controllable morphology. To date, the lowest reported temperature for growth of MWNT is 200°C, from benzene-thermal-reduction catalysis (BTRC), which uses chloride-containing precursors with *in situ* alkali metal-facilitated reduction (164).

Besides using dendrimers to encapsulate metallic nanoparticlar catalysts, dendrimers can be employed in enzyme catalysis. Recently, it has been demonstrated that amino acids can be assembled into catalytically functional peptide dendrimers. This peptide dendrimer approach aims at studying catalysis and selectivity arising from the interplay between amino acids within the dendrimer structure. A library of 21 different dendrimers has been synthesized and two dendrimers were found to catalyze the hydrolysis of 7-hydroxy-*N*-methyl quinolinium esters in water with significant rate enhancement and chiral discrimination. Two other dendrimers in the series catalyzed the hydrolysis of hydropyrene-trisulfonate esters. The rate enhancements obtained were up to 4000-fold, which compares well with catalytic peptides and most catalytic antibodies reported for similar esterolytic reactions. These experiments present the first examples of chemo- and stereoselective catalysis using peptide dendrimers (165).

The utilization of dendrimers for recoverable catalysts and reagents (166) and dendrimers in catalysis (167) have been thoroughly reviewed recently.

5.4. Dendrimer as Chelators. The high density of nitrogen ligands within PAMAM dendrimers and the possibility of attaching various functional groups, such as carboxyl, hydroxyl, etc, to their surface make PAMAM dendrimers particularly attractive as high capacity chelating agents for metal ions not only in catalytic applications but also as metal ion scavengers for waste remediation (168). Other surface chelators are under investigation. For example, catecholamides are powerful Lewis base chelators found in bacterial siderophores, sequestering agents for the hard Lewis acid Fe(III). Chelators related to catecholamides, including salicylamides, 1,2- and 3,2-hydroxypyridinonamides, and 2,3-dihydroxyterephthalamides are also good chelators for a variety of hard metal cations such as Fe(III), Pu(IV), and Gd(III). The high stability of metal complexes formed with these chelators makes them useful materials for metal separation technologies and waste remediation (169). Recent research seems to be focusing on Fe(III) and Cu(II) as model compounds for metal binding to dendrimers. Hydroxypyridinone hexadentate-terminated dendrimers have a high binding affinity for Fe(II) with $\log K = 34.8$, compared to $\log K = 30.6$ found for Fe(III) (170).

A family of iron-chelating dendrimers displaying carboxyl, catechol or 3-hydroxy-6-methyl-pyran-4-on ligands on their surfaces has been synthesized, and their stability has been studied by mass spectrometry (171). Removal of copper from contaminated sandy soil by use of PAMAM dendrimers of various generations and surface groups has been investigated. For example, >90% of copper initially sorbed in the soil was removed by use of similar to 66 bed volumes of 0.1% (w/w) of a generation 4.5 dendrimer with carboxylate surface at pH 6.0 (172). In another study, the use of Cu(II)-binding dendrimers in ultrafiltration (UF) applications has been studied. It was found that the dendrimers had a very low tendency to foul the commercially available regenerated cellulose membranes used for UF (173). Dendrimer-coated magnetic particles have been applied to radionuclide removal from nuclear waste by magnetic separation. Dendrimers carrying chelating groups for lanthanides and actinides on their surfaces were able to reduce the presence of europium and americium by factors 50–400 compared to particles without dendrimer coat (174).

6. Conclusions

The high level of control over the architecture of dendrimers, their size, shape, branching length and density, and their surface functionality, makes these compounds ideal materials in medical and technical applications, such as drug delivery, gene transfection, imaging, catalysis, and waste remediation. The active agents may either be encapsulated into the interior of the dendrimers or they can be chemically attached or physically adsorbed onto the dendrimer surface, with the option to tailor the properties of the dendritic carrier to the specific needs of the active material in its specific applications. Surface modified dendrimers themselves may act as nanodrugs against tumors, bacteria, and viruses. Recent successes in simplifying the synthesis of dendrimers such as the "lego" and "click" approaches have provided a vastly expanded variety of dendritic compounds, while at the same time reducing the cost of their production. This review of medical and technical applications of dendrimers clearly illustrates the potential of this new fourth architectural class of polymers and substantiates the high optimism for the future of dendrimers in these important fields.

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