Dendrimers in Oncology: An Expanding Horizon

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1. Introduction

Cancer is a killer disease second only to heart problems.[1] Though there is significant progress in the field of anticancer technology, we are still badly in need of a reliable cure for malignant growths.[2] At present, a variety of drug delivery approaches including polymer microcapsules and microspheres, liposomes, polymer conjugates, and nanoparticles are either FDA-approved or are in clinical development as cancer treatments.[3] The success of novel strategies for cancer therapy relies strongly on the development of reliable delivery devices capable of improving the therapeutic index of biologically active molecules. During the last few decades in particular, medical science has witnessed the exploration of several delivery devices,[4−7] and along with them a multitalented versatile star named "dendrimers" is now visible on the horizon.

Dendrimers are synthetic macromolecules with a tree-like well-defined branched structure.[8] Their peculiar architecture and flexibility in modifying it in numerous ways[9] has been an active area of research. Since their introduction[10,11] the unique characteristics of dendrimers have led to an exponential increase in the number of publications in this innovative field. Recently, progress has been made in the application of biocompatible dendrimers to cancer treatment, including their use as delivery systems for potent anticancer drugs such as cisplatin and doxorubicin.[12] Bifunctional polyamidoamine (PAMAM)-based dendrimers that selectively target cancer cells are described in an issue of *Chemistry and Biology*.[13]

Because of these efforts and continual research in the same interesting area, a vista overlooking a dendrimeric family with more than 100 compositionally different dendrimers with great potential for drug delivery has opened up.[1415−21] Applications have included solubility enhancement,[22−25] MRI contrast agents,[26−34] neutron capture therapy,[35−44] gene therapy,[45−50] drug delivery,[51−54] nanocomposites, [55−58] and photodyanamic therapy.[59−61] In the present review, we have summarized the work done with dendrimers in the field of cancer therapy, ranging from solubilization to hybrid dendrimer mediated targeting for cancer therapy (Figure 1,2).

2. Simple Dendrimers

2.1. Drug-Loaded Dendrimers as Nanovehicles

Since their introduction in the mid-1980s, this novel class of dendrimer architecture has been a prime candidate for host−guest chemistry.[8,20] Perhaps one of the main intriguing architectural functions of dendrimers relates to their containment properties. Initial studies of dendrimers as potential delivery agents focused on their use for noncovalent encapsulation of drug molecules.[62] This is based on physical entrapment, hydrophobic interactions, and ionic interactions.[31]

Dendrimers consist of three critical architectural domains: (i) a multivalent surface (nanoscaffolding), (ii) interior shells, and (iii) a core to which the dendrons are attached. The latter two domains present the nanoenvironments which are

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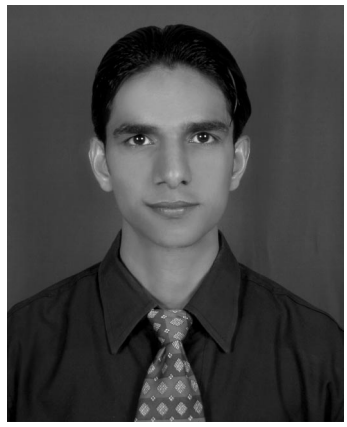

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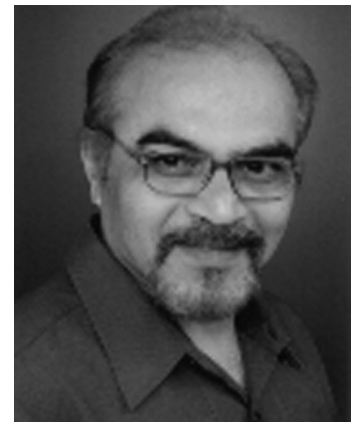

Narendra Kumar Jain was born in 1949. He earned his first degree in pharmacy in 1970 and his Ph.D. in 1978, both from University of Saugar (now known as Dr. Hari Singh Gour University) Sagar, India. He started his career as lecturer in 1973 and was promoted to Full Professor in 1986. Professor Jain's main research interests lie in hydrotropic solubilization of drugs, treatment strategies for *Helicobacter pylori*, controlled and novel drug delivery systems, and nanotechnology. He has published over 300 papers and review articles as well as 12 books. He is Chairman, Editorial Board of *Asian Journal of Pharmaceutics* and serves on the editorial board of few other journals. He received several awards for best papers from Indian Drugs Manufacturer's Association, M. L. Khorana Prize, Motan Devi Dandiya Prize for Best Research Paper in Pharmacy and Teacher of the year 2003 award of Association of Pharmaceutical Teachers of India.

protected from the exterior by the dendrimers' surface in the case of higher generation dendrimers. On the other hand, the interior is well-suited for host−guest interactions and encapsulation of guest molecules. These domains can be very easily tailored to impart specific purposes to the architecture. Meanwhile in the concept expressed as the "click in" mechanism,[63] wherein it has been suggested that the acid−base reaction between the dendrimer and the delivery agent with subsequent coulombic attractions pulls the guest into the dendrimer, while the hydrogen bonding keeps it bound to the target.[33] However it should be noted that the guest molecules were retained within the dendritic branching clefts by weak ionic interactions with interior protonated amide groups. Therefore, the inclusion complexes separated after deprotonation of the amide groups at pH less than 7.[64] This key principle characterizes the inherent property of dendrimers, which in other ways limit the release of drugs in relatively higher proportion at tumor sites, where pH lower to neutrality exists (Figure 3).

Grinstaff and co-workers used the known biocompatible monomers to develop several polyether-ester dendrimers and employed one composed of succinic acid and glycerol. They investigated the interior environment of poly(glycerol succinic acid) [PGLSA] dendrimers using the highly solvatochromic Reichardt's dye encapsulated within the nanostructure. The ^{1}H NMR of the encapsulated complex showed the existence of aromatic protons from Reichardt's dye in addition to the aliphatic protons of the dendrimer, and proton nuclear Overhauser enhancement (^{1}H NOESY) spectra for the complex showed a significant number of intermolecular NOE cross-peaks. These data revealed the close through-space proximity of the dye to the dendrimer along with the restricted motion of the encapsulated dye. To establish the potential use of this macromolecule as a drug delivery device, the poorly water-soluble anticancer drug 10-hydroxy camptothecin (10-HCPT) was encapsulated within a fourth generation (4.0G) PGLSA-dendrimer by the same group. Cytotoxicity assays of this drug with human breast cancer cells showed a significant reduction in the viability of tumor cells, demonstrating that the drug (10-HCPT) retains its anticancer activity even upon encapsulation within a PGLSA nanocarrier.[65−67]

It should be noted that the majority of anticancer drugs are hydrophobic in nature, and this property in particular creates major formulation problems. This drawback of anticancer drugs can be ameliorated by dendrimeric scaffolding, which can be used to encapsulate as well as to solubilize the drugs because of the capability of such scaffolds to participate in extensive hydrogen bonding with water (Figure 4). Hydrophobic interactions in general can play a vital role in this plethora of dendrimeric structures. Ooya et al. synthesized polyglycerol dendrimers (PGDs) with 4.0G and 5.0G, and used them to explore the effect of

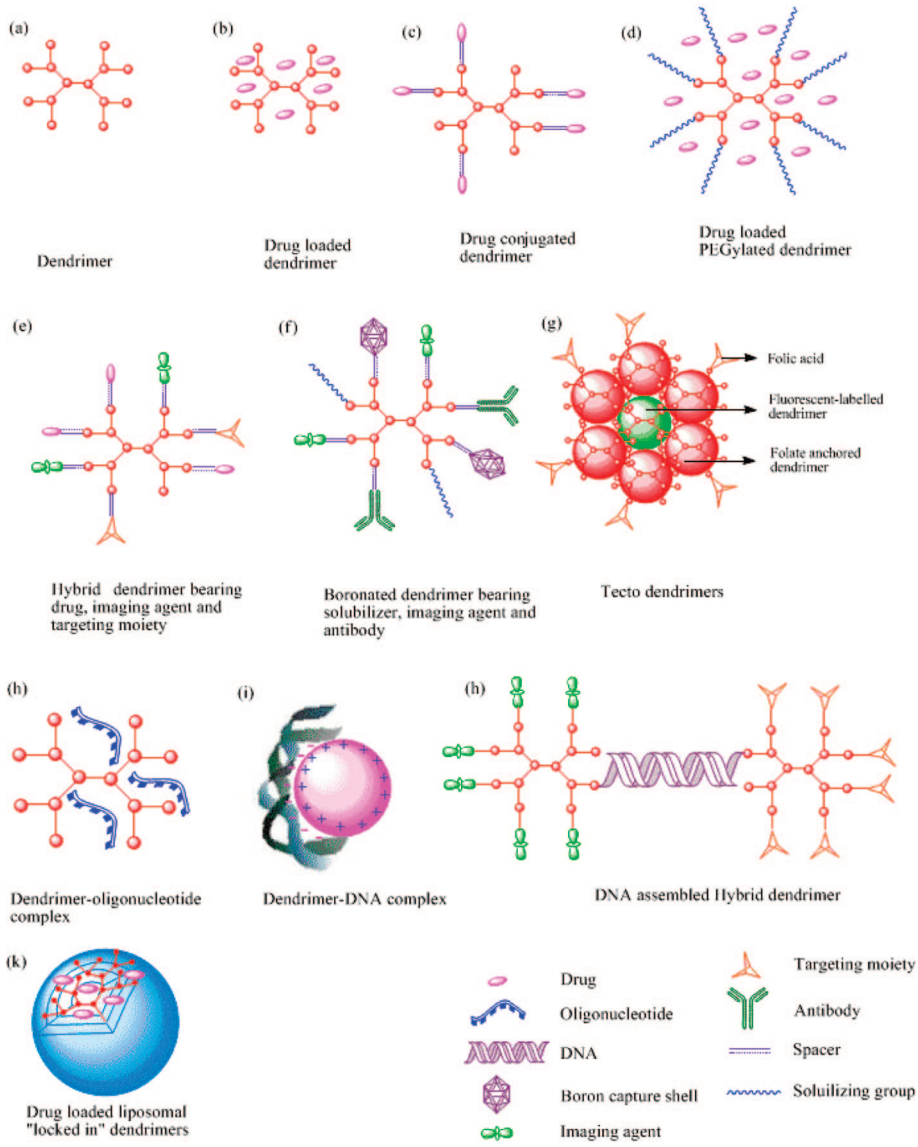

Figure 1. Schematic diagram showing different dendritic conjugates employed to date in cancer therapy and diagnosis.

dendritic architecture and generation on aqueous solubilization of paclitaxel, which is a poorly water-soluble anticancer drug. As expected, the increase in the water-solubility of paclitaxel by PGDs was found to be a function of the dendrimer generation. The ^{1}H NMR spectra of paclitaxel before and after mixing with PGDs suggested that the aromatic rings and some of the methyne groups of paclitaxel were surrounded by PGDs, which provided the basis for hydrotropic solubilization of the drug. The paclitaxel solubility in all the solutions of PGDs, even at concentrations below 10 wt %, was much higher than that of PEG_{400}, a frequently employed cosolvent and hydrotropic agent.[68]

Dow Chemical Company patented the antineoplastic dendritic polymer conjugates, which are useful for carrying antineoplastic agents to tumor sites. The anticancer moiety is encapsulated within the dendritic scaffold using an ionic charge shunt mechanism, whereby it interacts with the anionic functional groups on the surface of the dendritic polymer allowing the antineoplastic agent to be taken up by the scaffold through an association with the functional groups of the interior of the dendritic scaffold. This conjugate may be administered intravenously, orally, parenterally, subcutaneously, intraarterially, or topically in an amount effective in inhibiting tumor growth to an animal having a malignant tumor. The antineoplastic dendritic architecture demonstrated the high drug carrying capacity, good stability, and low toxicity as well as the good water solubility.[69]

Kohle, et al. studied the dendrimeric scaffold by taking a model compound, ibuprofen. Since this same concept of drug encapsulation is applied for all anticancer drugs, there is an urgent need to understand this work and its outcome. The NH_2-terminated 3.0 and 4.0G PAMAMs predominantly form a complex with the $-$COOH ion of ibuprofen because of ionic interactions, while the OH-terminated polyol appears to encapsulate ibuprofen. Up to 78 molecules of the model drug 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 construct was successful in transporting the model drug into lung epithelial carcinoma cells. In the complexed state, the model drug appeared to enter the cells at rates comparable to the pure dendrimer, which is significantly faster than for the free drug alone. More than 80% of complexed ibuprofen localized intracellularly in 1 h, whereas it took almost 3 h for 80% pure ibuprofen to reach the interior of the cell.[70]

Further, to explore the dynamics of the cellular entry of dendrimers, Kannan et al. employed the same drug as a

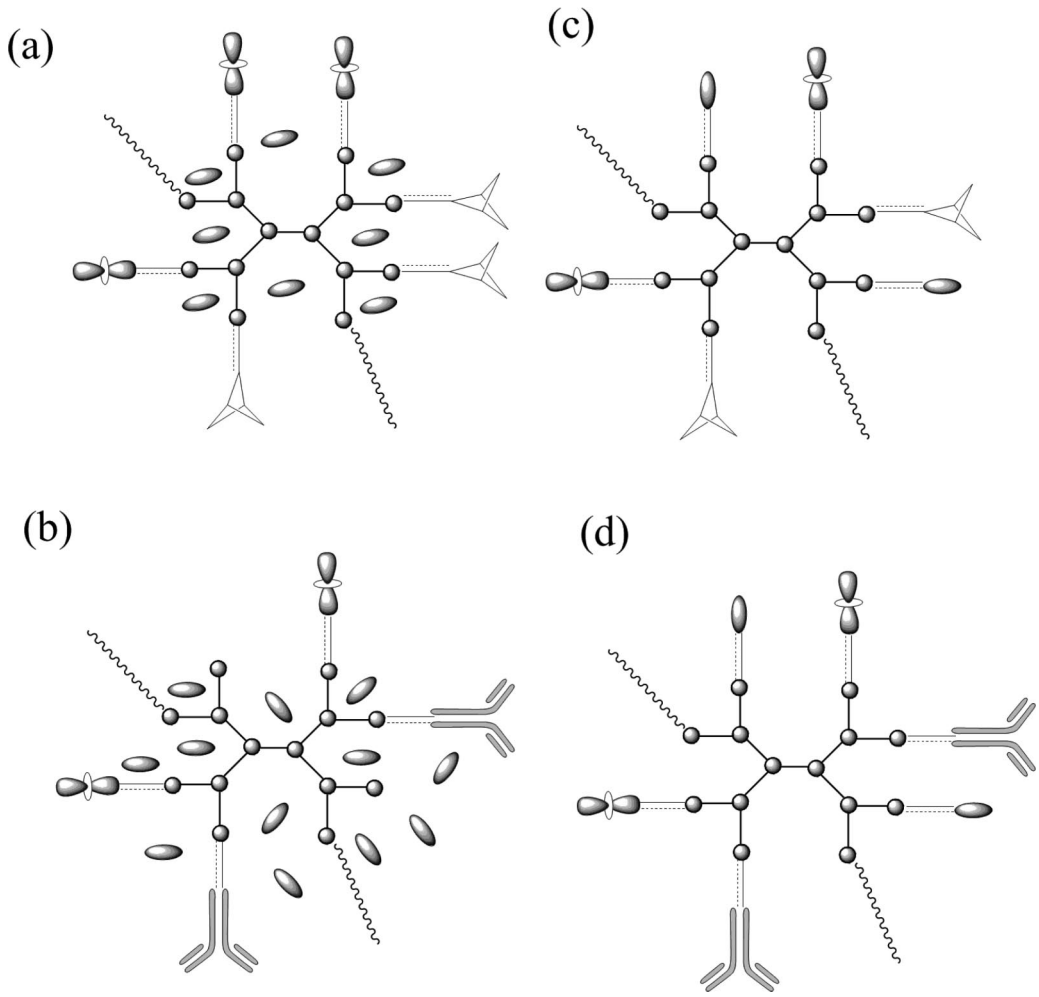

Figure 2. Schematic representation of future prospects in permutation and combination of dendrimers in cancer therapy and diagnosis: (a,b) bioactive-loaded, (c,d) bioactive-conjugated hybrid systems.

model construct for tracking dendrimer entry into A549 human lung epithelial carcinoma cells. The drug payload for the conjugates was found to be approximately 50% by weight with PAMAM−NH_2 dendrimers and was about 30% with polyol−OH and PAMAM−OH dendrimers. The PAMAM dendrimers with NH_2 and OH terminal functionalities appear to enter cells within 1 h, which is sooner than the hyperbranched polyol (OH functionality) requiring about 2 h. Cellular entry of PAMAM−NH_2 was detectable within 5 min. All branched polymers and their ibuprofen complexes entered A549 lung epithelial carcinoma cells rapidly when compared to the times required for entry of the unmodified drug.[71] In a recent follow up study, the same group covalently attached 58 molecules of ibuprofen to one molecule of fluoroisothiocynate (FITC)-labeled 4.0G PAMAM−OH dendrimer and investigated its cellular entry in human lung epithelial carcinoma A549 cells. Significant amounts of the conjugate entered the cell rapidly, within 15 min. Unlike the simple drug-loaded dendrimers, the covalently linked drug−dendrimer conjugates would be more stable in vivo, thus prolonging drug circulation and tissue delivery.[72]

Jevprasesphant et al. have evaluated the cytotoxicity, uptake, and transport mechanisms of PAMAM dendrimers and their relative surface-modification using monolayers of the human colon adenocarcinoma cell line, Caco-2. The permeation as well as cytotoxicity was found to increase with the concentration and generation of the dendrimers. The cytotoxicity of cationic dendrimers (2.0, 3.0, 4.0G) was greater than that of anionic dendrimers (2.5, 3.5G), but was significantly reduced by conjugation with lauroyl chloride. At 37 °C the apparent permeability coefficient (P_{app}) of cationic dendrimers was higher than that of anionic dendrimers. They concluded that the P_{app} values generally increased with the number of attached lipid chains. Furthermore, the P_{app} values for dendrimers and modified dendrimers were higher in the presence of ethylenediamine-tetra acetic acid, lower in the presence of colchicine, and higher at 4 °C than at 37 °C.[73] These data may serve as a guide for improving dendrimer-mediated permeation (Table 1).

2.2. Simple Dendrimers in Gene Transfection

Gene therapy focuses on the correction of genetic defects by transferring active genes into target cells.[81,82] The key to success in gene therapy specifically relies on exact and efficient delivery of genetic material to target cell populations. An ideal therapeutic DNA delivery vector would possess a high degree of target cell specificity, a high transfection efficiency, easy biodegradability, good stability, and a near baseline potential for the occurrence of toxicity and immunogenecity. In addition, it should be simple to design and synthesize DNA release and expression.[83] A number of delivery devices such as polyethylenimine (PEI)[84] and chitosans[85] have been designed as carriers for DNA molecules with these goals in mind. Viruses were the earliest gene delivery vectors, but the risks associated with them limit

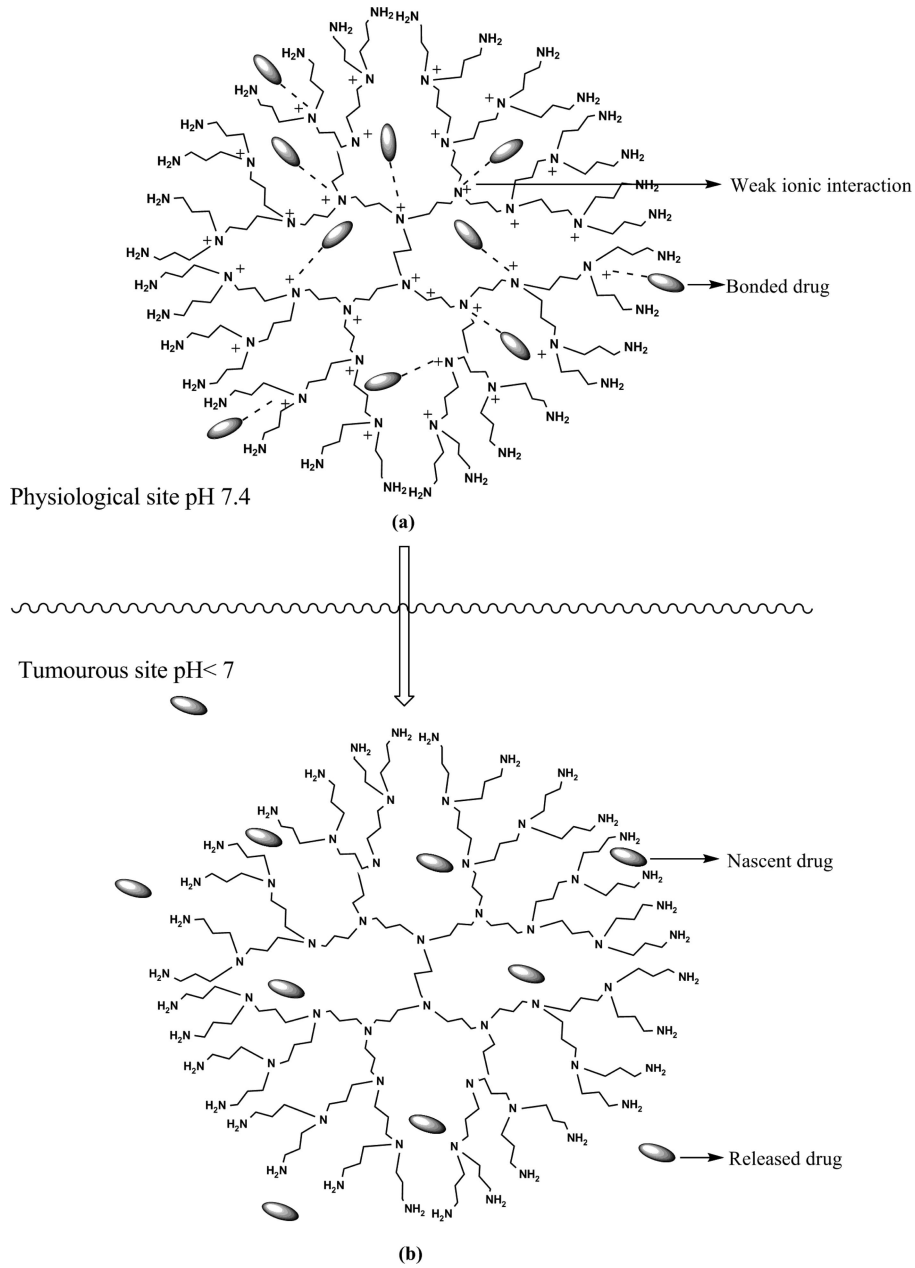

Figure 3. Mechanism of drug release following deprotonation from drug encapsulated dendrimers at tumorous site. (a) Protonated dendrimers showing retention of drug due to weak ionic interaction, pH 7.4. (b) Deprotonation of dendrimer at subneutral, pH < 7, followed by drug releasing event at tumorous site.

their carrying ability, especially since they can be strongly immunogenic because of their proteinaceous capsid. Such toxicities have been seen in numerous animal models.[86−88] In 1999 a patient participating in an FDA-approved gene therapy clinical trial died of respiratory and multiorgan failure, which was attributed to a lethal immune response to the adenovirus vector used to deliver the gene. This lead to temporary suspension of all gene therapy trials in the United States[89] and triggered the search for unmarked synthetic DNA delivery systems.

Many presently available vectors have widespread distribution, which precludes using them for site-specific targeting.[90] Numerous linear cationic lipids and cationic oligopeptides that possess the ability to form electrostatic complexes with DNA have been tested, but owing to their relatively high degree of cytotoxicity and low delivery efficiencies, such applications have been restricted.[91] Polymers like chitosan had inherently potent pharmacological properties (e.g., hypocholesterolemia) that made them very unsuitable for human use.[92] Several cationic polymers were synthesized, but their intrinsic drawbacks (e.g., low solubility, cytotoxicity, and low transfection efficiency) limited their clinical use as in vivo gene carriers.[93−95] In the same study branched-structure PEIs were recognized as being effective gene transfer agents,[84] but they were later found to be extremely cytotoxic by induction of apoptosis.[96] It is reported that (poly-L-lysine) PLL−DNA complexes undergo biodistribution into acidic lysosomes, which favors DNA degradation upon cellular internalization.[97] Apart from the immunogenicity and toxicity problems caused by their amino acid backbone,[98] the usage of such constructs is also limited by their nonspecific cell membrane binding.[99]

Using B16F10 murine melanoma cells, Seib et al. performed studies to compare binding, endocytic capture, and intracellular trafficing of linear and branched PEIs and cationic PAMAM dendrimers (2.0−4.0G). FITC−dextran

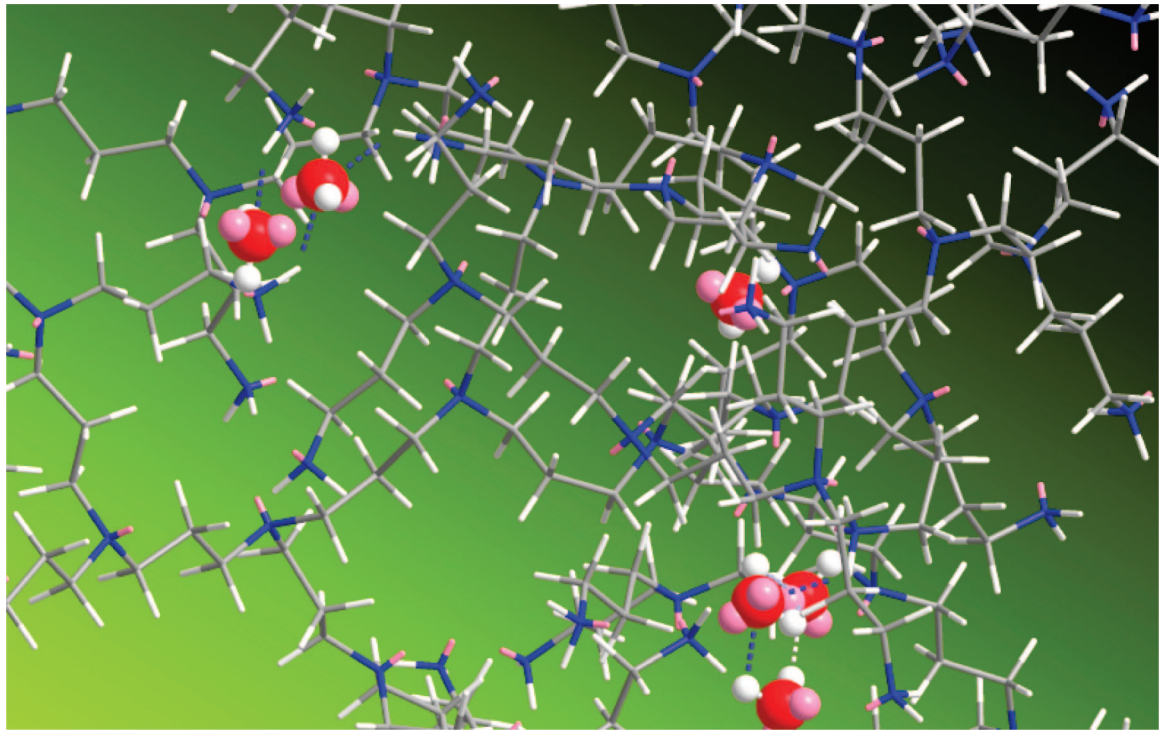

Figure 4. Molecular modeling support showing hydrogen bonding (dashed lines) between 5.0G PPI dendrimer (sticks) and water molecules (space fill); Chem3D Ultra 10.0 (CambridgeSoft). Jain, N.K. and co-workers, 2006 (unpublished report).

was used as a control for comparison. Flow cytometry showed that all of the cationic polymers were internalized by "adsorptive" endocytosis, with PAMAM 4.0G displaying the maximum rate of internalization (greater than that for branched PEI) at about 130-fold greater than the control FITC−dextran. Furthermore, the experiments suggested that the polycations interact with specific membrane component(s), which may regulate their cellular uptake route.[100]

At about the time of Seib's studies, dendrimer−DNA complexes formed as a result of ionic interaction between positively charged dendrimer and negatively charged DNA fragment came into use to transport DNA (Figure 5).

PAMAM dendrimers are a class of polycationic synthetic polymers that can be used for gene transfer.[101,102] Early studies were promising, showing that PAMAM dendrimers are significantly more efficient and less toxic than polylysine and can stabilize oligonucleotides within the cell.[31] There are some reports which suggest that lower generations also can be effective in gene delivery.[103] DeLong et al. showed that a 3.0G PAMAM dendrimer forms stable complexes with oligonucleotides. The 1:l and 20:1 complexes of dendrimer and oligonucleotide significantly increased the cellular uptake by about 3−4 and 50 folds, respectively.[76] These results were in contrast to those of Tomalia and co-workers,[75] who reported that efficient complexation and facilitated cellular uptake of DNA occurred only when higher generation PAMAM dendrimers were used.

PAMAM dendrimers have now been established as an efficient class of polycationic synthetic polymers used for gene transfer.[101,102,104] The 3-dimensional spherical structure of dendrimers offers control of the molecule in terms of generation and degree of branching. This control can produce polymer particles with a very low degree of polydispersity, which is a striking advantage over other classes of polymers such as PLL that generate extremely polydisperse particles. It is noteworthy that low polydispersity can lead to reproducible gene delivery and a clinically reliable formulation.[101,102] Superfect and Polyfect (Qiagen, Valencia, CA) are commercially available 6.0G-branched activated dendrimer formulations for in vitro gene delivery. The cationic amino acid residues in the polymeric structure of PAMAM dendrimers can help in DNA condensation and endosomal release.[77] Because of the presence of protonated primary amine groups on their surface, these highly branched dendrimeric scaffolds possess a highly positive charge density that is responsible for both the ionic condensation of DNA and binding to the negatively charged cancerous cell surface. Protonated residues may also provide endosomal buffering and thus protect DNA from lysosomal degradation[48,105] (Figure 6).

Ackermann et al. broadened the applicability of dendrimers as gene transfection agent in comparison to the well-established theory of lipofection. This study examined whether tumor and fibroblast cell lines established from Ewing's sarcoma patients could be transfected with the IL-2 gene. Starburst dendrimers (Superfect), a safe transfection reagent, were chosen for a transfection study, and the most favorable conditions for gene transfer were evaluated. ELISA was used to measure the concentration of IL-2 in the supernatant of transfected cells. Ewing's sarcoma cell lines after dendrofection yielded higher IL-2 levels than that attained by lipofection. In contrast to lipofection, expression of IL-2 increased with time and peaked later. In one of the three veteran fibroblast cell lines, transfection using Superfect yielded elevated IL-2 levels. IL-2 production was in general lower in fibroblasts as compared to Ewing's sarcoma cell lines. The most favorable high efficiency of transfection should be the most promising for clinical studies on Ewing tumors immunotherapy.[78]

Recently, Tziveleka et al. fuctionalized the 4.0G PPI dendrimer either partially or completely with guanidinium groups. In the partially functionalized dendrimers the remaining toxic primary amino groups of the dendrimers were

Table 1. List of Simple Dendrimers Employed in Cancer Technology

type of scaffold	bioactive studied	purpose/outcomes of study	reference
PAMAM	DNA	protonated residues may also provide endosomal buffering and thus protect DNA from lysosomal degradation	Bielinska et al.[48]
PAMAM	oligonucleotides	PAMAM dendrimers are significantly more efficient and less toxic than polylysine and can stabilize oligonucleotides within the cell	Haensler and Szoka[62]
PGLSA-dendrimers	10-HCPT	to encapsulate, increase the solubility and assess the retention of anticancer activity by 10-HCPT upon encapsulation.	Grinstaff and co-workers.[65−67]
polyglycerol dendrimers	paclitaxel	to explore the effect of dendritic architecture and generation on aqueous solubilization of paclitaxel	Ooya et al.[68]
PAMAM	ibuprofen (model drug)	drug complexation, in vitro release and cellular entry of dendrimers; using model drug into lung epithelial carcinoma cells	Kolhe et al.[70]
PAMAM	ibuprofen (model drug)	to explore the dynamics of cellular entry of dendrimers and track its entry into A549 human lung epithelial carcinoma cells	Kannan et al.[71]
PAMAM	_	to assess the cytotoxicity, permeation, and transport mechanisms of PAMAM dendrimers and its relative surface-modification using monolayers of the human colon adenocarcinoma cell line	Jevprasesphant et al.[73]
PAMAM/PSS construct	doxorubicin	selective encapsulation of drug into the dendrimers to enhance the localization within the shell of the capsule	Khopde and Caruso.[74]
PAMAM	DNA	to study complexation and facilitated cellular uptake of DNA	Kukowska-Latallo et al.[75]
PAMAM	oligonucleotides	demostrated that a 3.0G PAMAM dendrimer forms stable complexes with oligonucleotides.	DeLong et al.[76]
PAMAM	DNA	to verify that cationic amino acid residues in the polymeric structure of PAMAM dendrimers can help in DNA condensation and endosomal release	Merdan et al.[77]
PAMAM	IL-2 gene	examined IL-2 gene transfection in tumor and fibroblast cell lines established from Ewing tumor patients.	Ackermann et al.[78]
PPI	^{32}P labeled oligonucleotide	to deliver triplex-forming oligonucleotide in breast, prostate and ovarian cancer cell lines.	Santhakumaran et al.[79]
PSCD, NSCD		to access the in vivo biodistribution for differently charged PAMAM dendrimers in B16 melanoma and DU145 human prostate cancer mouse tumor models	Nigavekar et al.[80]
PAMAM, linear PEI, FITC dextran		PAMAM displayed maximum rate of internalization in melanoma cells, as compared to other polymeric constructs under investigation	Seib et al.[100]
guadinylated PPI	DNA	guadinylation significantly enhances the transfection efficiency.	Tziveleka et al.[106]
arginine PAMAM	DNA	compared the gene delivery potency of PAMAM and PAMAM−Ag in carcinoma cells.	Kim et al.[107]

reacted with propylene oxide to afford the corresponding hydroxylated derivatives. These guanidinylated dendrimers were interacted with plasmid DNA affording the corresponding dendriplexes. It was found that complete replacement of primary amino groups with the hydroxylated moieties resulted in complete loss of transfection efficiency. In contrast, guanidinylation of the parent dendrimer results in significant enhancement of its transfection efficiency. The completely guanidinylated dendrimer exhibited the best transfection efficiency under all circumstances studied. This was attributed to the enhanced penetrating ability of the guanidinylated dendrimers due to the accumulation of the guanidinium group at the dendrimeric surface. However, they also found that the derivative with 12 guanidinium groups exhibited the least toxicity. This reduction in toxicity was due to the decrease in the number of external primary amine groups along with the presence of hydroxylated moieties at the surface. It is obvious that the toxicity of completely guanidinylated dendrimers (DAB-G32) limits its gene transfer potency.[106]

Kim et al. designed a novel type of arginine-rich dendrimer with a structure based on PAMAM. The polymers were found to self-assemble electrostatically with plasmid DNA forming nanometer-scale complexes. From dynamic light

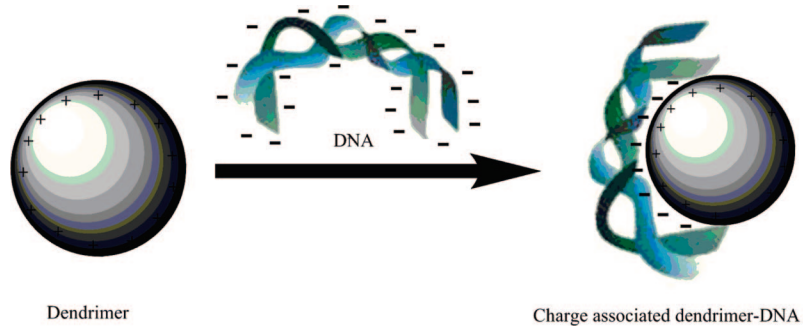

Figure 5. Schematic diagram showing formation of dendrimer−DNA complex mediating charge interaction between positively charged dendrimer and negatively charged DNA.

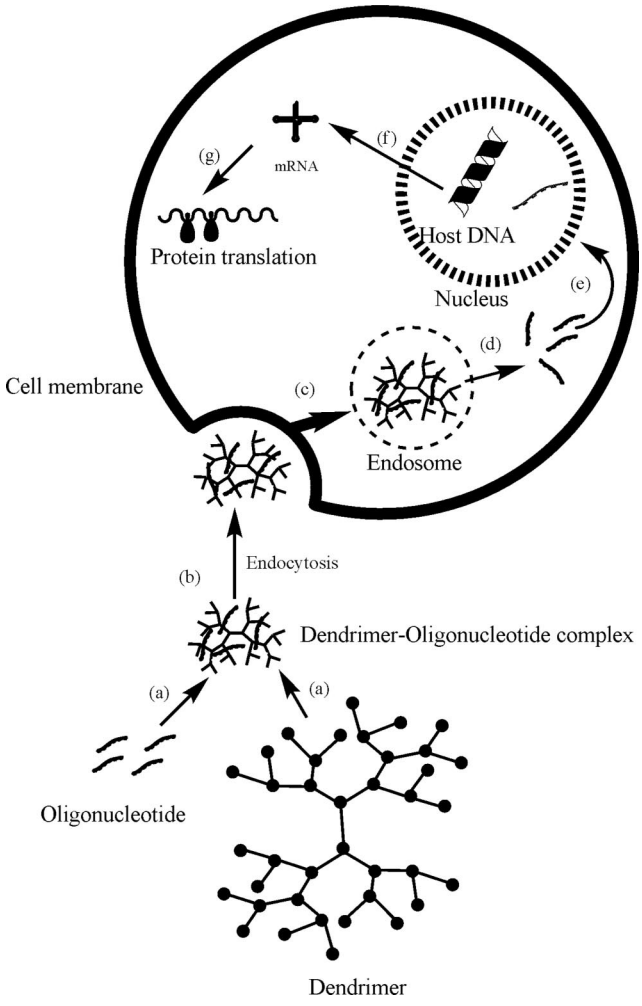

Figure 6. Schematic representation of events involved in dendrimer-mediated oligonucleotide delivery: (a) formation of stable electrostatic complex between dendrimer and oligonucleotid; (b) endocytosis mediated uptake of complex; (c) endosomal destabilization of electrostatically assembled dendrimer−oligonucleotide complex; (d) oligonucleotide release; (e) nuclear uptake of olgonucleotide and its subsequent replication with host DNA; (f) release of mRNA as biosignal; (g) occurence of protein translation.

scattering experiments, the mean diameter of the polyplexes was found to be around 200 nm. The complex composed of PAMAM−Arg/DNA showed increased gene delivery potency compared to native PAMAM dendrimer and PAMAM−Lys.[107] Furthermore, the transfection efficiency assessed in human liver carcinoma HepG2 cells and their outcomes showed a great degree of correlation with other findings.[108,109]

Santhakumaran et al. reported the use of polypropylene imine (PPI) dendrimers for delivering a triplex-forming oligonucleotide in breast, prostate, and ovarian cancer cell lines using ^{32}P-labeled antisense oligonucleotide (ODN). Analytical reports suggested that complexing of oligonucleotides with dendrimer markedly increased its stability. Dendrimers were found to enhance the uptake of oligonucleotides by ∼14-fold in MDA−MB-231 breast cancer cells as compared to the uptake of control oligonucleotides. Dendrimer effects depended on their molecular weight, with 4.0G having the maximum efficacy. A similar increase in ODN uptake was found with MCF-7 (breast), SK−BR-3 (breast), LNCaP (prostate), and SK−OV-3 (ovarian) cancer cells. Uptake was also found to be increased with increasing dendrimer concentration; this reached a maximum at ∼0.05 mM concentration for 4.0G dendrimer and then leveled off. The growth-inhibitory effects of the oligonucleotides were also found to be significantly increased upon complexing with 4.0G dendrimers. Complexing of ^{32}P-labeled oligonucleotides with all five generations of PPI dendrimers enhanced the internalization of the ^{32}P-labeled oligonucleotides as compared with the uncomplexed oligonucleotides.[79]

In the same field a major effort was made by investigators at the University of Michigan to assess the in vivo biodistribution for differently charged PAMAM dendrimers in the murine B16 melanoma and human DU145 prostate cancer tumor models. This group had synthesized the neutral surface charged dendrimers (NSCD) and positive surface charged dendrimers (PSCD) by using 5.0G PAMAM dendrimer and ^{3}H-labeled acetic anhydride. These constructs were then tested for in vivo performance by intravenous injection, and their biodistribution was determined via liquid scintillation counting of tritium in tissue, urine, and feces. Simultaneously, the mice were also monitored for acute toxicity. Dendrimers cleared rapidly from the blood, with deposition peaking at 1 h for most organs and stabilizing from 24 h to 7 days postinjection. Maximal excretion occurred via urine within 24 h postinjection. Neither of the dendrimers showed acute toxicity. They found localization of both PSCD and NSCD to major organs and tumors. They also reported that changes on the surface of polycationic PAMAMs modified their biodistribution. PSCD deposition into tissues was higher than NSCD, though the biodistribution trend is similar. The highest levels were found in lungs, liver, and kidney, followed by those in tumor, heart, pancreas, and spleen, while lowest levels were found in brain. These nanoparticles could have future utility as systemic biomedical delivery devices.[80]

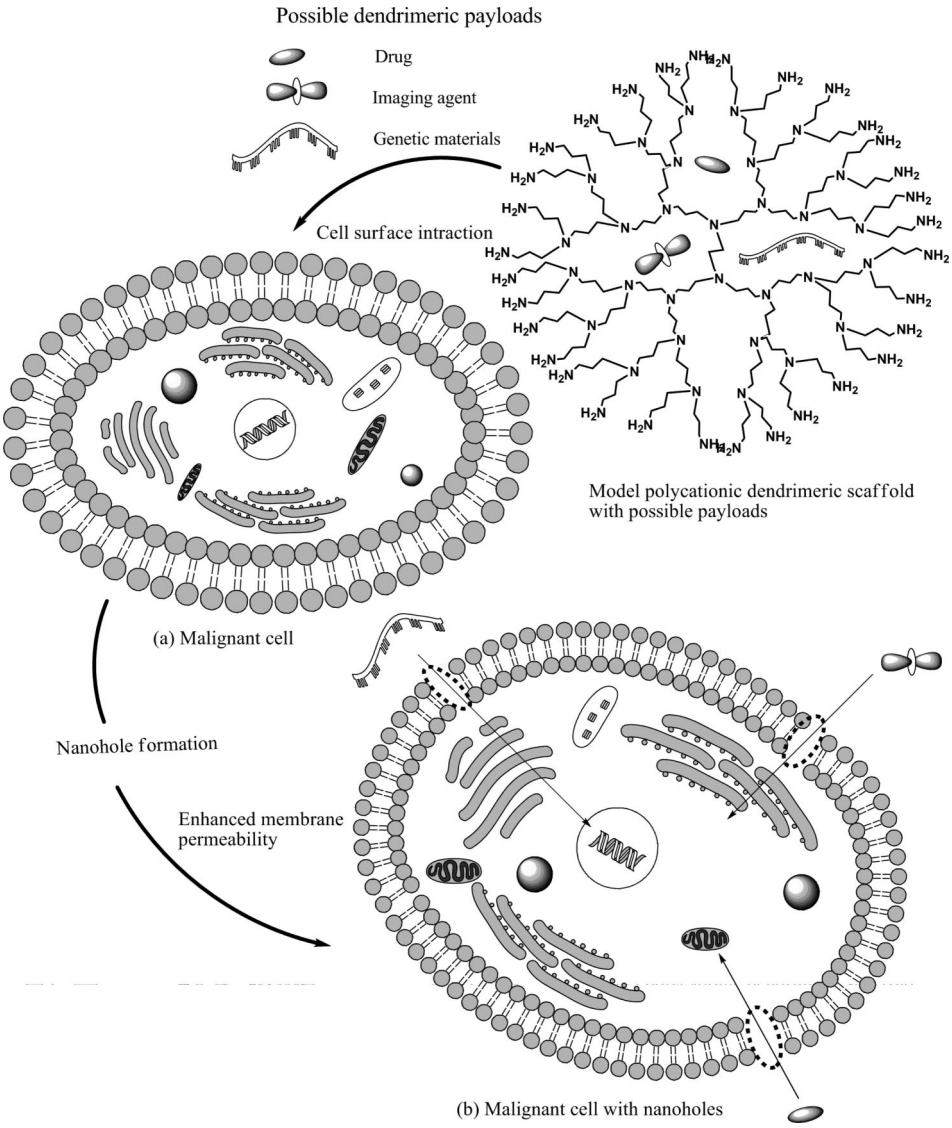

Figure 7. Schematic showing interaction of polycationic dendrimers with cells: nanoscale hole formation and enhanced membrane permeability. (a) Malignant cell in usual state; (b) malignant cell with nanoholes, which possibly mediate enhanced cellular uptake.

The studies of Hong et al. supported the hypothesis that polycationic polymers induce the formation of transient nanoscale holes in the living cells, and these nanoholes allow a significantly enhanced molecular exchange across the cell membrane[110] (Figure 7). Bayele et al. reported a new class of lipidic-dendrimers for protein transduction into cultured cells, which also may be capable of intracellular protein delivery. They also reported that these dendrimers could be used for gene and drug delivery. Such a finding not only accelerates the field of protein therapeutics,[111] but also suggests that in cancer technology permutations and combinations of these scaffolds could be used to transport large payloads across the membrane (Figure 1).

Until now, many researchers have employed simple unimolecular dendritic systems because of their several universally accepted advantages over conventional polymeric micelles (Table 1). On the other hand, this architecture does have its disadvantages, with the major ones being uncustomized release and hemolytic cytotoxicity. In some cases harsh conditions are required[112] whereas in others the encapsulated drug is not well retained and escapes rapidly.[113,114] Covalent drug-conjugation on the surface dendritic scaffold is expected to show a considerable decrease in its cytotoxicity[115,116] because of attainment of selective drug release pattern.

3. Drug Conjugated Dendrimers in Cancer Therapy

It is clear now that polymeric drug conjugates have several advantages over free drugs, among them being increased plasma half-life, decreased drug resistance, and linkage-tuned drug release. Several synthetic and natural polymers have been tested in the past decade for targeting tumor cells, and several crucial theoretical implications have been developed in the past, but the actual synthesis of such compounds remains challenging.[117,118]

An alternative strategy for utilizing dendrimers as anticancer drug carriers is to make the most of their well-defined multivalent structure in covalent attachment of drug molecules to their periphery. The release of the bioactive can be tuned by applying the concept of site selective degradable spacer between the drug and the peripheral groups of dendrimer. Moreover the drug loading can be tuned by varying the number of groups on the dendrimer's periphery. 5- Fluorouracil (5-FU) has potent antitumor activity, but it

also has very toxic side effects. Several partially successful attempts at reducing its toxicity have been made.[119−121] In studies aimed at improving the outcomes obtained with 5-FU, Zhou et al. reported the synthesis of a series of dendritic polymers (0.5−5.5G) starting from 1,4,7,10-tetraazacyclododecane, a cyclic tetraamine core. The dendrimers were first acetylated and then reacted with 1-bromoacetyl-5-FU to form dendrimer−FU conjugate. These conjugates were observed to be highly water-soluble and to release free 5-FU at a slow rate with a concomitant reduction of its toxicity only upon incubation with phosphate-buffered saline (PBS; pH, 7.4) at 37 °C.[122] This observation should be compared with the work of Liu et al.[113] and Kojima et al.,[114] who encapsulated the drug molecule inside the dendrimer and found that the encapsulated drugs were not well retained and were released relatively rapidly.

Duncan and co-workers have studied dendrimers for their potential in delivery of anticancer agents. They conjugated 3.5G PAMAM dendrimers with cisplatin to yield a dendrimer−platinate (dendrimer−Pt; 20−25 wt % platinum) which was highly water-soluble and released platinum slowly in vitro. In this way cisplatin, a potent anticancer drug with significant toxicity and poor water-solubility, was tailored to have increased solubility, decreased systemic toxicity, and selective accumulation in solid tumors. The dendrimer−Pt complex (**1**) and cisplatin (**2**) were equipotent in vivo. Both **1** and **2** were equiactive against intraperitoneal L1210, but against intraperitoneal B16F10 melanoma, a high dose of Dendrimer−Pt given intraperitoneally showed activity, whereas cisplatin did not. Increased efficiency was reported with dendrimer–platinum complexes in the treatment of subcutaneous B16F10 melanoma, where cisplatin was inactive. Dendrimer−Pt in solid tumor tissue showed a 50-fold increase in the area under the curve as compared to that of pure cisplatin because of the enhanced permeability and retention (EPR) effect. The considerably reduced toxicity from this novel antitumor approach was reported to be around 3−15-fold smaller than from pure cisplatin.[123,124]

Wiener et al. conjugated the free amines of PAMAM dendrimers to the chelator 2-(4-isothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid (TU−DTPA). One of the Dendrimer−metal chelate conjugates had 170 gadolinium ions bound, which greatly exceeded the number bound to other macromolecular agents reported in the literature. The dendrimer gadolinium polychelates have enhancement factors, that is, the ratio of the relaxivity per Gd(III) ion to that of Gd(III)−diethylenetriamine pentaacetic acid, of up to 6. These factors are more than twice those observed for analogous metal−chelate conjugates formed with serum albumins, polylysine, or dextran. The report suggested this new and powerful class of contrast agents could be of great potential in magnetic resonance imaging (MRI).[125]

Bulte et al. have investigated dysprosium [Dy]−DOTA−PAMAM (5.0G) dendrimers as potential macromolecular T2 contrast agents. For this investigation, 5.0G ammonia-core PAMAM dendrimers were linked to the bifunctional ligand *p*-SCN−Bz−DOTA. The T1 relaxivity values for Dy−DTPA, Dy−DOTA, and the Dy−DOTA-based dendrimer were independent of field strength and had values between 0.12 and 0.20 mM^{-1} s^{-1}. At lower fields (0.05−0.1 T), 1/T2 was identical to 1/T1. However at higher fields, 1/T2 increased quadratically with field strength with a strong dependence on temperature. The field-dependent component of 1/T2 was up to three times greater for the Dy−DOTA-based dendrimer than for the single chelate molecules.[126]

Bryant et al. conjugated PAMAM dendrimers (5.0, 7.0, 9.0, and 10.0G) with the bifunctional chelate 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*,*N*‴-tetraacetate. Gadolinium(III) ion was added to the macromolecules, and the 1/T1 and 1/T2 were measured at 3°, 23°, and 37 °C. At 20 MHz and 23 °C, the 1/T1 ion relaxivity increased from 30 mM^{-1} s^{-1} for 5.0G to 35 mM^{-1} s^{-1} for the 7.0G PAMAM dendrimer−DOTA−Gd, reaching a plateau at 36 mM^{-1} s^{-1} for the 9.0G and 10.0G dendrimers. A similar plateau was observed for 1/T2 with values of 36 mM^{-1} s^{-1} for 5.0G, 42 mM^{-1} s^{-1} for 7.0G, and 45 mM^{-1} s^{-1} for the 9.0G and 10.0G dendrimers. The 1/T1 and 1/T2 relaxivities decreased with temperature for each generation of dendrimer studied, and it was suggested that slow exchange of bound water molecules with the bulk solvent limits the relaxivity. In such circumstances, an increase in the rotational correlation time of the macromolecules associated with higher generations of dendrimer does not result in significant increases in the ion relaxivity. Although the ion relaxivity does not increase, the total molecular relaxivities were amplified from 2880 to 66960 mM^{-1} s^{-1} in going from the 5.0G to the 10.0G dendrimer.[32]

The prolonged retention of large macromolecular MRI contrast agents is a major limitation for their clinical use. Kobayashi et al. evaluated small dendrimer-based MRI contrast agents for their pharmacokinetics, whole-body retention, and dynamic MRI in mice in comparison to Gd−[DTPA]−dimeglumine. They reported that smaller dendrimer-based MRI contrast agents were more quickly excreted by the kidneys and were also capable of visualizing vascular structures better than Gd−DTPA due to less extravasation. In addition, unlike Gd−DTPA, they rapidly accumulated in renal tubules and thus also can be used to visualize renal structural and functional damage. Diaminobutane (DAB) dendrimer-based agents were reported to show more rapid clearance from the body than PAMAM dendrimer-based agents with the same numbers of branches.[34]

The utility of acid sensitive linkages had been demonstrated by Greenfield et al.[127] for adriamycin immunoconjugates. The conjugate was reported to be stable at the physiological pH of 7.4, but found to undergo hydrolysis upon uptake of the polymers by endocytosis and subsequent trafficking to mildly acidic subcellular organelles such as the endosomes and lysosomes. Keeping these reports in mind, Ihre et al. [128] reported the design and synthesis of dendritic polyester systems based on the monomer unit 2,2-bis (hydroxymethyl) propanoic acid as a possible versatile drug carrier. The potent anticancer drug doxorubicin was attached via a pH-sensitive linkage, thus demonstrating the feasibility of using these polyester dendritic structures to produce polymer-drug conjugates capable of delivering the drug to a chosen low pH cancerous site (Figure 8).

Wang et al. had reported the synthesis of an efficient star-copolymer for the delivery of doxorubicin by conjugating semitelechelic poly [*N*-(2-hydroxypropyl)-methacrylamide] (ST−PHPMA, arm) macromolecules with PAMAM dendrimers (2.0, 3.0, 4.0G). The terminal −COOH groups were activated with *N*-hydroxysuccinimide followed by the attachment of doxorubicin to the arms of the polymer via a biodegradable peptide spacer. This resulted in a dense structure exhibiting slow enzyme-mediated drug release from

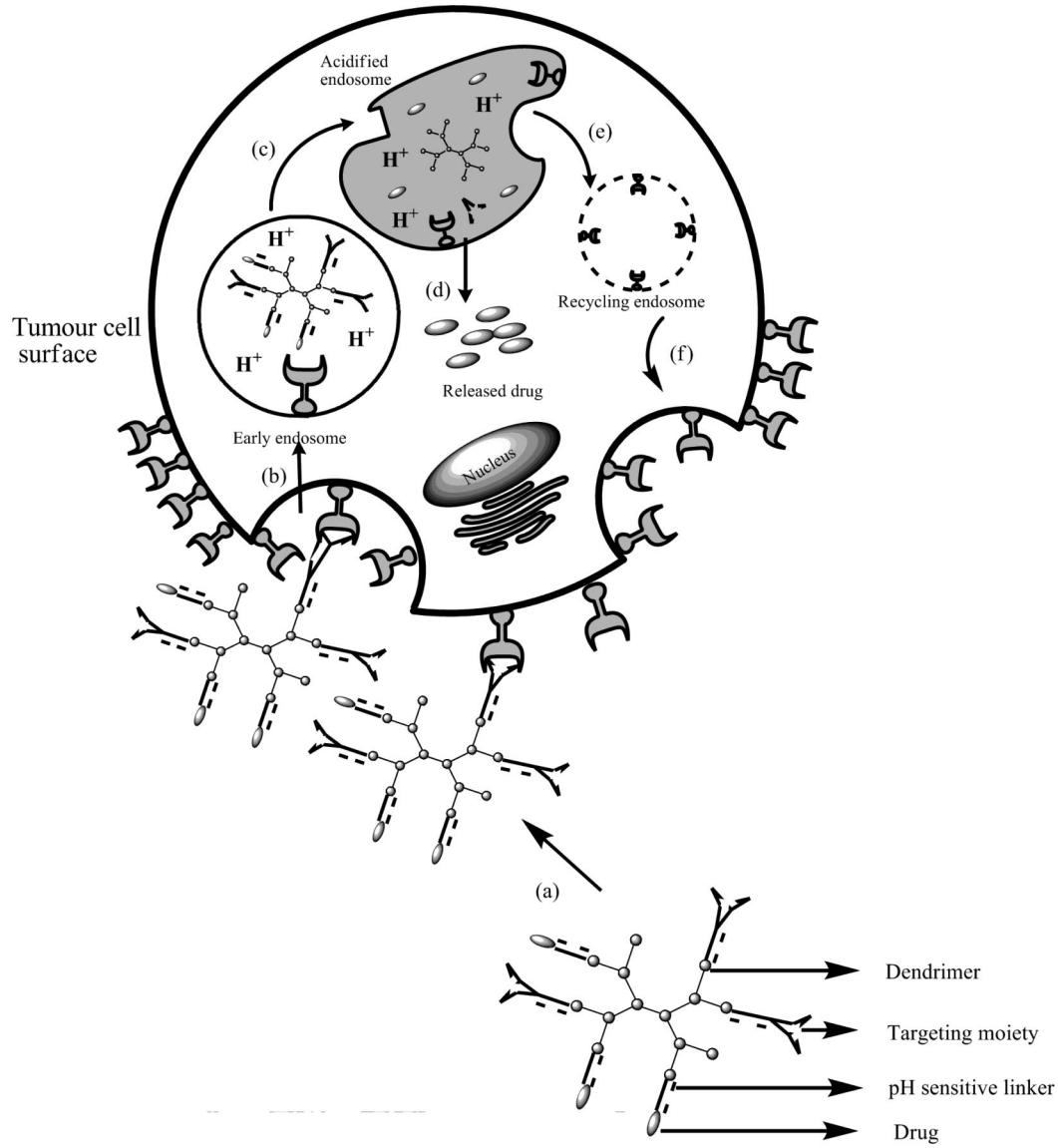

Figure 8. pH responsive drug release from targeted dendrimeric conjugate possessing pH sensitive linker: (a) receptor association, (b) endosomal uptake, (c) rupture of acid-sensitive linkage between dendrimer and drug, pH sensitive linker ensures selective endosomal release (pH less than 7), (d) drug release following burst effect, (e, f) receptor regeneration.

the conjugate with concomitant diminished cytotoxicity. Cytotoxicity of the DOX-containing star-copolymer was determined using the human A278 ovarian carcinoma cell line, and it was reported to be far better than DOX-containing linear counterparts.[129]

Studies have also been performed to evalute the complexation/conjugation ability of these dendrimeric scaffolds toward a wide variety of drugs such as adriamycin (ADR) and methotrexate (MTX) for chemotherapy,[114] epinephrine for the treatment of shock, and solu-medrol for the treatment of asthma. Several covalent and noncovalent conjugates of small cancer chemotherapeutic agents are now in more advanced levels of clinical trials, and several other polymer constructs, including dendrimers, are in early clinical trials.[130] More recently Bellis et al. reported a method for obtaining dendrimers with peripheral cisplatin moieties by the reaction of the free amine dendrimers and potassium tetrachloroplatinate(II). For this they used the three generations of PPI dendrimers modified with α,γ-diaminobutyric acid moieties.[131]

Khandare et al. studied methylprednisolone (MP) as a model construct in A549 human lung epithelial carcinoma cells. They compared two methods to produce methylprednisolone-4.0G PAMAM conjugates for mediating drug delivery. In method 1, PAMAM dendrimers were first coupled to glutaric acid (GA) as spacer and then further conjugated with MP to obtain PAMAM−GA−MP conjugates. This scheme yielded a lower conjugation ratio of MP, most likely because of lower reactivity and steric hindrance at the crowded dendrimer periphery. In method 2, this steric hindrance was overcome by first preparing the MP−GA conjugate, which was then attached to the PAMAM dendrimers. Using method 2, they were successful in conjugating 12 molecules of MP with the dendrimer, corresponding to a payload of 32 wt %. In addition, conjugates were further labeled with fluorescein isothiocyanate (FITC) to evaluate the dynamics of cellular entry on A549 human lung epithelial carcinoma cells. Fluorescence and confocal microscopy images showed that the conjugate was localized mostly in the cytosol. MP−GA−dendrimer conjugate showed pharmacological activity comparable to that of free MP. This model defined future guidelines for conjugating a high payload of anticancer drugs to a dendrimeric surface employing a suitable spacer.[132] Such conjugates can poten-

Table 2. List of Drug-Conjugated Dendrimers Employed in Cancer Therapy

type of scaffold	bioactive studied	purpose/ outcomes of study	reference
cyclic core (1,4,7,10-tetraazacyclododecane) dendrimer	5-flurouracil	enhance water solubility, achieve selective slow release and reduce toxicity	Zhou et al.[122]
PAMAM	cisplatin	solubility enhancement, decrease systemic toxicity and selective tumor accumulation.	Malik et al.[123] Duncan et al.[124]
polyester	doxorubicin	selective delivery of drug via a pH-sensitive linkage	Ihre et al.[128]
ST−PHPMA−PAMAM star-copolymer	doxorubicin	achieve enzyme-mediated slower drug release; diminishing drug cytotoxicity.	Wang et al.[129]
PAMAM	ibuprofen (model drug)	study transport of drug into lung epithelial carcinoma cells.	Kolhe, et al.[70]
PPI	cisplatin	investigate peripheral drug conjugation.	Bellis et al.[131]
PAMAM	methylprednisolone (model construct)	explore the role of spacer in drug loading and bioactivity in A549 lung epithelial carcinoma cells.	Khandare et al.[132]
flurosceine labeled phosphodiester and phosphorothioate dendrimer	oligonucleotides	investigate the intracellular uptake.	Skobridis et al.[134]

tially be further conjugated with an appropriate targeting moiety to deliver the drugs to a specific cancer type.

Hussain et al. selected an antisense oligonucleotide (ODN) complementary to an accessible region of the epidermal growth factor receptor mRNA (EGFR). They successfully conjugated nine ODN molecules to a single dendrimer and evaluated the ability of this conjugate to deliver and down-regulate EGFR expression in cancer cells. In vitro RNase H-mediated cleavage tests confirmed that covalently conjugated antisense ODNs in the dendrimer conjugate were able to hybridize and cleave the array-defined hybridization target site within the EGFR mRNA without the need for ODN dissociation from the conjugate. The conjugate showed improved stability toward serum nucleases as compared to the free ODNs. The cellular uptake of ODN−dendrimer conjugates was up to 4-fold greater than nude ODN in cancer cells. In cell culture, ODN−dendrimer conjugates were effective in the inhibition only of that cancer cell growth which was correlated with a marked reduction in EGFR protein expression.[133]

Recently Skobridis et al. studied the influence of the dendrimer on cellular uptake of the oligonucleotides by using fluorescein-labeled phosphodiester and phosphorothioate 2.0G dendrimer-conjugates. Fluorescence experiments revealed that covalent attachment of lipophilic dendrimers resulted in a substantial increase in the cellular association of oligonucleotides, and subsequently an intracellular accumulation of the oligonucleotide conjugates. The fluorescence was significantly enhanced (i.e., by 11-fold) by the fluorescein-labeled phosphodiester−oligonucleotide−dendrimer conjugate. The effect was still more pronounced for the fluorescein-labeled phosphorothioate oligonucleotide−dendrimer conjugate, which gave a 15-fold enhancement of uptake. The report suggests that the dendrimeric part of the conjugates acts as a lipophilic anchor and facilitates the penetration of the oligonucleotides through the cellular membrane.[134]

But, apart from this, there are still numerous problems associated with dendrimers. The most common of these are drug escape allowed by the relatively open configuration and hemolytic toxicity from peripheral $-NH_2$ groups.[135,136] Covalent conjugation was tried (Table 2) to avoid frequent drug escape, but cytotoxicity still remained an issue of concern. PEGylation has been used to make dendrimers less susceptible to drug seepage as well as to decrease their hemolytic toxicity. Recent research has focused on the

development of biocompatible dendrimers, which will hasten the era of conjugation of anticancer bioactives within PEGylated dendrimers.

4. PEGylated Nanocarriers in Cancer Therapy

In cancer therapy, PEGylated dendrimers are a class of nanocarriers which are capable of effectively delivering high drug payloads relatively unharmed to attack cancer. PEGylated dendrimers not only drastically augment drug loading and solubilization, but also eliminate the naked dendrimeric scaffold drawbacks of hemolytic toxicity, uncontrolled drug outflow, macrophageal uptake, short half-life, etc. PEGylation of dendrimers appreciably improves their kinetic stability and makes them useful for the extended delivery of bioactive species. PEGylation can also improve targeting to the active sites of action with reduced immunogenecity, antigenecity, and toxicity by shielding the dendrimers against destructive mechanisms of the body. Often this approach helps in avoiding situations such as are seen in gene therapy.[135] The use of PEGylated dendrimers in cancer treatment technology has been studied extensively, and this plethora of studies can be discussed more systematically by grouping them under the following headings.

4.1. PEGylated Dendrimers: A Way to Achieve Solubilization and Controlled Release of Chemotherapeutics

A poorly water-soluble anticancer candidate manifests several in vivo consequences: hampered bioavailability, raised probability of food effect, unfinished release from the formulation, and also greater interpatient variability. Poor water solubility also often accounts for in vitro obstacles such as limited choices of delivery technology, complicated dissolution testing, and/or poor in vitro: in vivo correlation. Poorly soluble but effective anticancer drugs such as cisplatin and MTX motivated the development of new delivery devices to overcome the obstacles on the way to their solubilization. A number of technologies have been applied for solubilizing anticancer entities, ranging from traditional particle size reduction via comminution, spray drying, and micellar solubilization to cyclodextrin-mediated inclusion complexes. There is an extensive literature reporting cyclodextrin- and micelle-mediated solubility enhancement, but the high cost and nephrotoxicity of the former and the disruption by dilution of the micellar structure of the latter limit the use of these techniques. At present, PEGylated dendrimers are an innovative class of dendrimers with the additional advantage of attached PEG-chains, which not only enhances their solubilization ability but also augments surface crowding and thereby enables controlled release from the dendrimeric scaffold.

An early model demonstrating the advantage of the stepwise synthesis and the controlled multivalency of PEGylated dendrimers for drug delivery was put forward by Liu et al., wherein it was possible by using a careful synthetic approach to attach both hydrophobic drugs and polyethylene oxide (PEO) moieties to the dendrimer periphery in a controlled manner.[136] Hence this approach can be considered a hybrid of drug-conjugated[122] and PEGylated dendrimers.[137] In addition, Liu et al. explored the potential of dendritic unimolecular micelles for drug delivery using both the container and sustained drug releasing properties of such systems.[113]

Ooya et al. studied the effects of polymeric architecture on the solubilization and release of paclitaxel, a poorly water-soluble drug. In their work they investigated the effect of the density of ethylene glycol chains on the solubility enhancement of paclitaxel. This group synthesized the poly(oligo ethylene glycol) methacrylate (OEGMA), star shaped poly(OEGMA), and polyglycerol 3.0, 4.0, and 5.0G dendrimers (Figure 9). Poly(OEGMA) increased the paclitaxel solubility, but a much more significant effect was observed with the five-arm star poly(OEGMA). The aqueous solubility produced by 10% star-shaped poly(OEGMA), and by 3.0, 4.0, and 5.0G polyglycerol dendrimers, respectively, were 130-, 270-, 370-, and 434-fold greater than the unenhanced paclitaxel solubility in water. These data are sufficient to conclude that polyglycerol dendrimers are much more effective in increasing the paclitaxel solubility than are the others under study. This is likely due to the increased local density of the ethylene glycol unit. Even with relatively similar molecular weight (M_w: 1690) and concentration (50 wt %) of the 3.0G dendrimers to PEG_{2000} (M_w: 2000), the paclitaxel solubility was raised 11-fold over that of PEG_{2000}. Polyglycerol dendrimers dissolved in water at high concentrations without significantly increasing the viscosity and, at 80 wt %, were found to increase the solubility of paclitaxel 10000-fold.[138] This proposed star dendritic polymer could be modified further in the future to serve as a useful tool for both oral and parenteral delivery of paclitaxel and other poorly water-soluble drugs.

In a follow-up study, Ooya et al. synthesized 4.0, 5.0G dendrimer having the same architecture and used this to investigate the effect of dendritic architecture and generation on the aqueous solubilization of paclitaxel. The paclitaxel solubility in all the solutions of polyglycerol dendrimers, even below 10 wt %, was much higher than that in PEG_{400}, which is commonly employed as a cosolvent or a hydrotropic agent. The increase in paclitaxel solubility was found to be a function of dendrimer generation. ^{1}H NMR spectra of paclitaxel before and after mixing with polyglycerol dendrimers (PGDs) in D_2O suggested that the aromatic rings and some methyne groups of paclitaxel were surrounded by PGDs, hence providing an excellent alternative mode for the hydrotropic solubilization of a poorly soluble drug.[68]

With the aim of achieving both solubilization and sustained release benefits, Kojima et al. synthesized a dendrimeric construct by combining the chain ends of 3.0 and 4.0G PAMAM dendrimers with poly(ethylene glycol) monomethyl ether via a urethane bond. The anticancer drugs MTX, a practically water insoluble drug, and adriamycin, a hydrophobic entity, were encapsulated within the hydrophobic interior of the PEGylated PAMAM dendrimer combination. The authors observed that the ability of the system to encapsulate these anticancer entities improved with improving dendrimer generation and chain extent of PEG grafts. Among the methoxy PEG (MPEG) attached dendrimers, the highest ability was achieved with the 4.0G-$MPEG_{2000}$, which could retain 6.5 adriamycin or 26 MTX molecules per dendrimer molecule. It was felt that adriamycin was solubilized and complexed on the chain surface of MPEG, while for MTX, the encapsulation efficiency of an acidic drug was increased by a greater electrostatic interaction arising from acid−base feedback between MTX and dendrimer (Figure 10). The MTX-loaded PEG dendrimers released the drug slowly in an aqueous solution of low ionic strength. However, in isotonic solutions, MTX and Adriamycin were readily

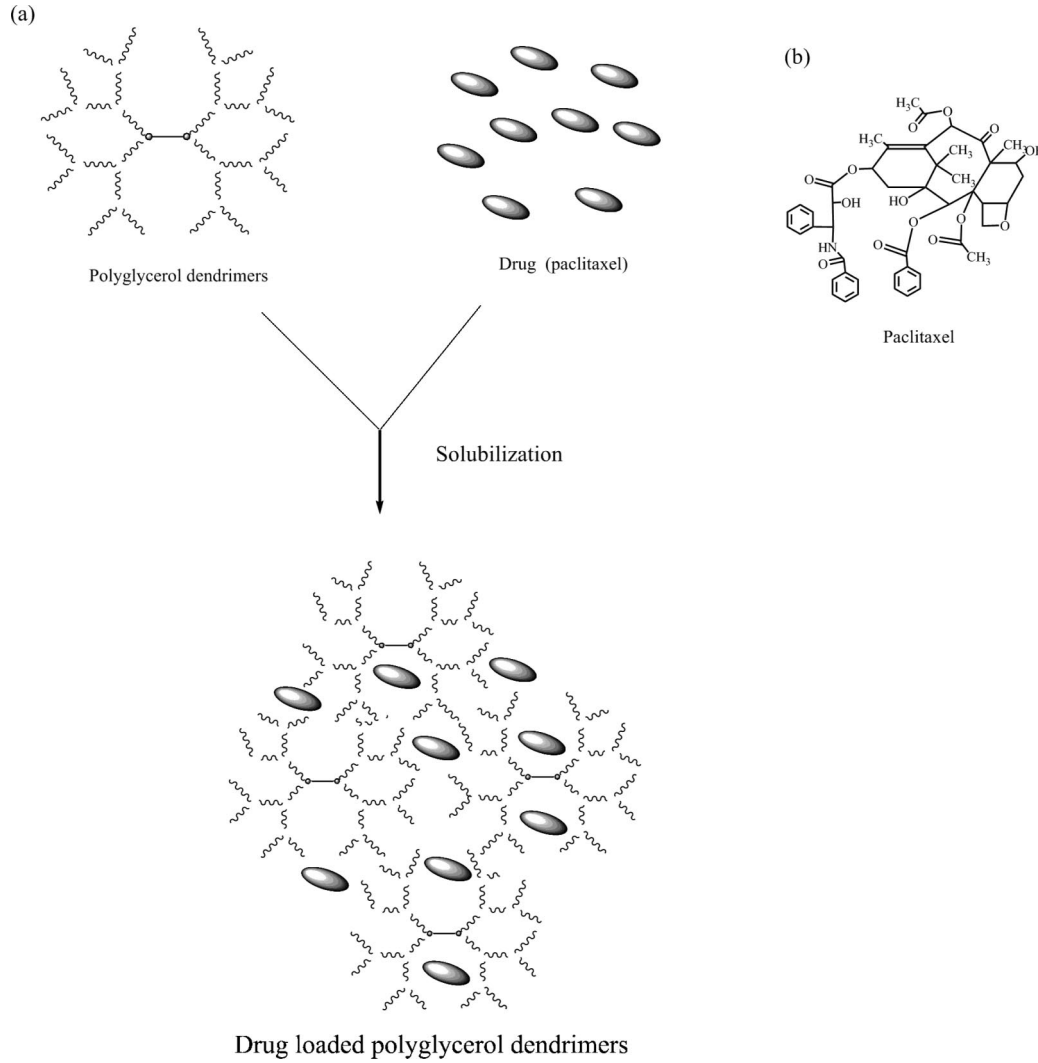

Figure 9. (a) Schematic showing loading and solubilization of paclitaxel by polyglycerol dendrimers; (b) structure of paclitaxel.

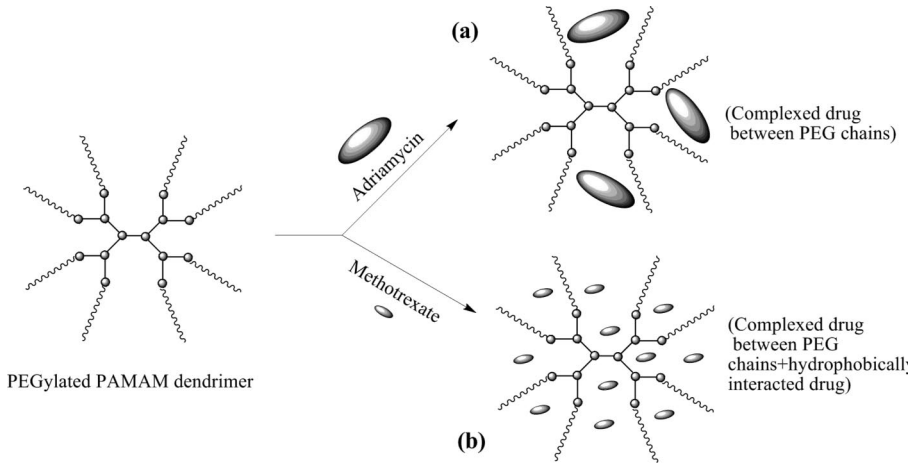

Figure 10. Schematic diagram showing drug loading in PEGylated dendrimers: (a) due to complexation of drug between PEG chains of dendrimer; (b) both due to existence of hydrophobic intraction owing to high acid−base feedback between drug and dendrimer as well as drug complexation between PEG chains of dendrimer.

released. Since drug release is accomplished by dialysis, targeted delivery would be difficult to attain, but sustained release would be easier to achieve. Furthermore, as the encapsulation via the dendrimer varied appreciably depending on the drug and the dendrimer configuration, this method would be quite tricky to make universal for all drugs.[114]

Apart from drug attachment, dendrimers also provide an entry into various new polymer architectures with the potential to provide some extraordinary check as drug delivery devices. As a case in point, Gillies and Frechet reported the design and preparation of new polyester dendrimers and poly(ethylene oxide) hybrid systems for drug

delivery and related therapeutic applications along with studies of their accumulation in solid tumors. These systems consisted of covalently attached polyester dendrons, where one dendron provides multiple functional handles for the attachment of therapeutically active moieties, while the other is used for attachment of solubilizing poly(ethylene oxide) chains. These new carriers are nontoxic and biodegradable in vitro, and biodistribution studies in vivo have shown that carriers with a molecular weight of more than 40000 are generally long circulating with half-lives greater than 24 h. The carrier is excreted at a slower rate into the urine by glomerular filtration, probably as a consequence of its decreased flexibility and ability to diffuse through pores relative to linear polymers. Substantial levels of long circulating bow-tie polymers accumulated in subcutaneous B16F10 solid tumors via the EPR effect, thus establishing a promising claim for these carriers in cancer treatment know-how.[139]

In view of the fact that macromolecules conjugated with polyethylene glycol acquire higher hydrophilicity, thus resulting in a longer half-life in circulation and lower immunogenicity, Margerum et al. applied the idea of PEGylated dendrimers to develop magnetic resonance imaging (MRI) contrast agents employing 2.0 and 3.0G PAMAM dendrimers. They synthesized the macrocycle 1-(4-isothiocyanatobenzyl) amido-4,7,10-triacetic acid-tetraazacyclododecane (D03A−bz−NCS) and coupled it to the terminal amine sites of starburst PAMAM dendrimers (*n*-SBDs) thus creating macromolecular polychelates. Gadolinium ion was also added to the dendrimer polychelates. The resulting complex (*n*-BD−GdD03As) was water-soluble and monodisperse. There was an increase in blood elimination half-life with molecular weight ranging from 11 min for 3-SBD−(GdD03A), (M_w: 22 kDa) to 115 min for the 5-SBD−(GdD03A), (M_w: 61.8 kDa), when studied in the rat model. The seven-day liver retention increased from 1 to 40% over the same molecular weight range. They also studied the effects of grafting PEG moieties onto *n*-SBD−GdD03A polychelates. Blood elimination half-lives increased significantly (range 33−1219 min), and the seven-day liver uptake was also decreased to 1−8% of the injected dose. These results suggest that the addition of covalently bound PEG to the *n*-SBD−GdD03A surface significantly improves the biological performance of the contrast agent.[140]

Later, Kobayashi et al. synthesized two novel conjugates for MRI contrast agents from 4.0G PAMAM dendrimers, 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriamine pentaacetic acid (TU-DTPA), and either one or two PEG molecules with a molecular weight of 20000 Da to yield PEG2−PAMAM−(TU−DTPA−Gd) and PEG1−PAMAM−(TU−DTPA−Gd) conjugate. They then evaluated their pharmacokinetics, excretion, and vascular MRI contrast agent properties as compared with those of the non-PEGylated counterparts. PEG2−G4D (TU−DTPA−Gd) conjugate remained in the blood for a significantly longer time and showed less accumulation in the liver and kidney than the other two preparations (Figure 11). In conclusion, the major positive effects of PEG conjugation on an MRI contrast agent constructed with PAMAM were prolonged retention in the circulation and decreased accumulation in the organs.[141] Furthermore, these results suggest that PEGylation will be an effective strategy for improving the in vivo performance (Table 3).

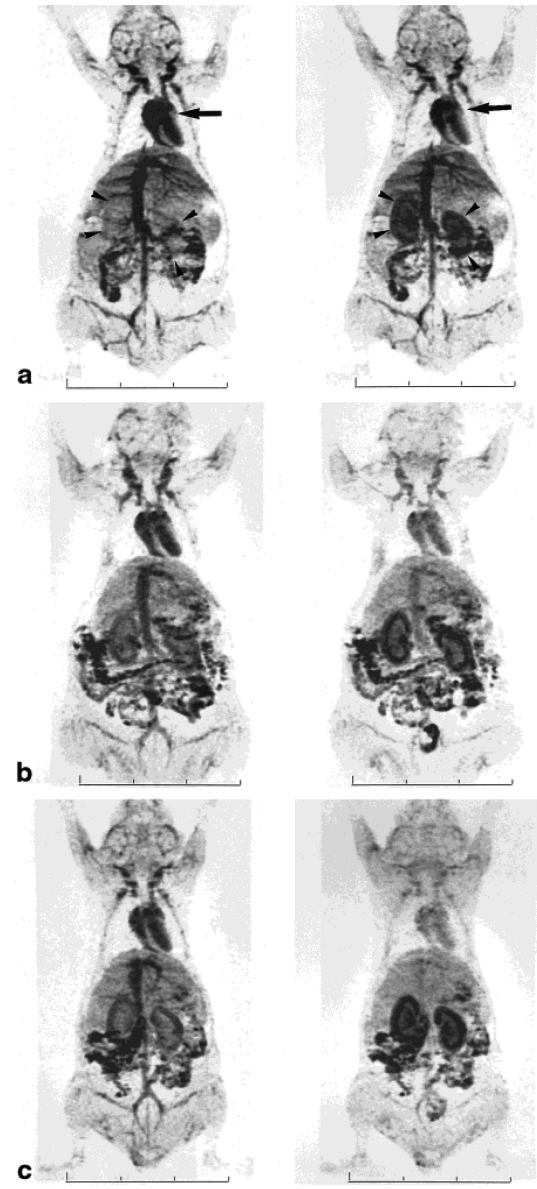

Figure 11. Whole-body 3D-micro-MRI of mice injected with 0.033 mmolGd/kg of (a) PEG2−G4D−(TU−DTPA−Gd)$_{62}$, (b) PEG1−G4D−(TU−DTPA−Gd)$_{63}$, and (c) G4D−(TU−DTPA−Gd)$_{64}$. The images, which are negative displays with higher intensity darker than lower intensity, were obtained at 2 min (left) and 10 min (right) postinjection. MIPs are shown. Similar images from all mice in the same group injected with same preparations were obtained. Darker blood (arrows) and brighter kidneys (arrowheads) were shown on both early and delayed images with (a) PEG2−G4D−(TU−DTPA−Gd)$_{62}$ compared to those with (c) G4D−(TU−DTPA−Gd)$_{64}$. The images with (b) PEG1−G4D−(TU−DTPA−Gd)$_{63}$ appeared intermediate between (a) PEG2−G4D−(TU−DTPA−Gd)$_{62}$ and (c) G4D−(TU−DTPA Gd)$_{64}$. Adapted with permission from ref 141. Copyright 2001 International Society for Magnetic Resononance in Medicine.

4.2. PEGylated Dendrimers: In Maintenance of in Vivo Stability

Dendrons based on aspartic acid units and arabinofuranosil cytosyne (Ara-C) conjugated via its amine group by various linkers including amides and carbamates were prepared by Choe et al. This strategy improved the in vivo stability and blood residence time of the drug and increased its stability. It should be noted that in contrast to PEGylation of active bioactive moiety, PEGylated drug loaded systems have a number of advantages including retention of bioactivity,

Table 3. PEGylated Dendrimers Employed against Cancer

type of scaffold	bioactive studied	purpose/outcomes of study	reference
PEGylated PAMAM	MTX/adriamycin	solubilization and sustained release benefits.	Kojima et al.[114]
PEGylated PAMAM	1-(4-isothiocyanatobenzyl) amido-4, 7,10-triacetic acid-tetraazacyclododecane and gadolinium	effect of PEG grafting on biological half-life.	Margerum et al.[140]
PEGylated PAMAM	TU−DTPA	assess blood residence and biodistribution pattern.	Kobayashi et al. [141]
star-shaped polyOEGMA; PGDs	paclitaxel	to investigate the effect of the density of ethylene glycol chains on the solubility enhancement.	Ooya et al.[138]
PGDs	paclitaxel	solubilization was more in case of PGDs as compared to PEG_{400}, a frequently used solubilizing agent.	Ooya et al.[68]
dendrimers based on melamine	–	cationic dendrimers were found to be more cytotoxic and hemolytic than anionic or PEGylated dendrimers.	Chen et al.[142]
PEO−dendrimer hybrids	Ara-C	to improve in vivo stability, blood residence time and drugs stability.	Choe, Y.H. et al. [143]
PEO−dendrimer hybrids	Ara-C	to enhance drug loading, in vivo stability and residence time.	Schiavon et al. [144]
PEO−dendrimer hybrid	doxorubicin	to have a long circulating, in vivo stable, pH responsive drug releasing carrier.	Padilla De jesus et al.[145]
PEGylated PAMAM dendrimer	5-fluorouracil	to reduce hemolytic toxicity and rate of drug release; along with enhanced solubilizing and loading capacity	Bhadra et al.[137]
either polylysine or polyester dendron	Nile Red (model construct)	the rate of release showed strong correlation with the rate of acetal hydrolysis (pH responsive hydrophobic group) which in turn often controls the release rate	Gillies et al.[146]
PEO hybrid		to develop a polymer drug delivery. It showed selective accumulation in solid tumors via EPR effect.	Gillies et al.[139]

bioenvironmental protection heading up this pool with dendrimers.[143] Schiavon et al. reported synthetic strategies for conjugating Ara-C via amino groups to the carboxy groups of a dendron. But the basic problem with the system was the low loading efficiency of the polymer, and solving this problem was attempted by functionalization of the hydroxyl functions of PEG with a bicarboxylic amino acid to yield a tetrafunctional derivative.[144]

As a general rule, high molecular weight molecules undergo slower renal filtration.[147] High molecular weight polymers (>20000 dalton) have been widely used as soluble drug carriers to improve drug targeting and therapeutic efficacy; dendritic polymers are one of the outstanding candidates. Hence, one move toward increasing the half-life is to make the dendrimer larger. Pang reported polyethylene oxide (PEO) to be a biocompatible polymer.[148] One hybrid implementation of this idea was made by Padilla De Jesus et al., who covalently conjugated doxorubicin via a hydrazone linkage to a high molecular weight 3-arm PEO−dendrimer hybrid. Their goal was to have a long circulating in vivo stable conjugate with its drug release controlled by pH. As expected, drug release from this conjugate was more rapid when exposed to a pH < 6. The cytotoxicity of the doxorubicin−polymer conjugate against multiple cancer lines in vitro was found to be reduced though not eliminated completely, thus indicating that some amount of active doxorubicin was also escaping from the drug polymer conjugate under physiological conditions. These results showed that the serum half-life of doxorubicin attached to a high molecular weight polymer was significantly increased, and also that, as compared to free doxorubicin, not much doxorubicin−polymer conjugate is accumulated in vital organs.[145]

Gillies and co-workers employed hybrids of PEO and either a polylysine or polyester dendron with attached pH responsive hydrophobic acetal groups on the periphery of the dendrimeric scaffold. At mildly acidic pH, loss of hydrophobic groups upon acetal hydrolysis triggers micellar disruption followed by payload release. To demonstrate the potential of these systems for controlled release, the release of Nile Red as a "model payload" was examined. At pH 7.4, the fluorescence of the construct encapsulating Nile Red was relatively constant, indicating it was retained in the micelle, but at pH 5, the fluorescence decreased, which was consistent with its release into the aqueous milieu. The rate of release showed strong correlation with the rate of acetal hydrolysis, which in turn often controls the release rate.[146]

In this context Okuda et al. have synthesized dendritic poly(L-lysine)s (DPKs), dendritic poly(L-ornithine)s (DPOs), and PEGylated sixth generation lysine dendrimers (KG6), and then evaluated their physicochemical properties as well as biodistribution characteristics. PEGylation of KG6 caused great changes in particle size, ζ-potential, blood retention, and organ distribution, which indicates that PEGylation is a useful strategy for improvement of the biodistribution characteristics of dendrimeric molecules. The information provided by this study will be helpful for the development of future drug delivery systems using amino acid dendrimers as safe drug carriers.[149]

In a follow-up study the authors synthesized a sixth generation lysine dendrimer and two PEGylated derivatives thereof and evaluated their biodistribution characteristics in both normal and tumor-bearing mice. The intact KG6 showed rapid clearance from the blood stream and nonspecific accumulation in the liver and kidneys. In contrast, the PEGylated derivatives showed a better retention in blood and a lower accumulation in organs, depending on the degree of PEGylation. Besides this, PEGylated KG6 with a high degree of modification was accumulated effectively in tumor tissue via the EPR effect. This succession of effects suggests that the PEGylated lysine dendrimers may be a useful base material for a clinically applicable tumor-targeting drug carrier device.[150]

4.3. PEGylated Dendrimers: Toward Augmentation of Biocompatibility of Anticancer Drugs and the System

From the above, it should be clear to everyone that dendrimers possess great potential as drug delivery devices for cancer chemotherapy. But before proceeding to the design of the next generation, the biocompatibility and toxicity of dendrimers as well as the chemotherapeutic effect of loaded dendrimers should be fully understood.

Bhadra et al.[137] from our research group set out to develop and explore the use of PEGylated PAMAM dendrimers for the delivery of 5-fluorouracil (5-FU). This PEGylated system showed low hemolytic toxicity and drug release rate along with enhanced loading and solubilizing capacity. Upon PEGylation of the dendrimers, the hemolysis of RBCs declined significantly, possibly due to inhibition of the interaction of RBCs with the charged quaternary ammonium ion in the presence of PEG chains.[151] The blood level of the system was prolonged so that it was detectable for up to 12 h. The average release rate for the PEGylated dendrimeric system was found to be 0.679%, which was nearly 1/6th of that from non-PEGylated systems. Such systems were found to be a suitable vehicle for the extended delivery of anticancer drugs in vitro as well as in vivo.[137]

Chen et al. prepared a library of six surface-modified dendrimers based on melamine but with variations in the chemistry of the surface groups: amine, guanidine, carboxylate, sulfonate, phosphonate, and PEGylated. These were evaluated for hemolytic potential and cytotoxicity. Cationic dendrimers were found to be more cytotoxic and hemolytic than anionic or PEGylated dendrimers. The authors also reported that when the PEGylated dendrimer was assayed for in vivo acute toxicity in mice, it showed no toxicity, lethality, or abnormalities in blood chemistry.[142]

Thus these nanocarriers have been proved to be efficient in reducing drug leakage, hemolytic toxicity, and renal filtration while raising in vivo stability and biocompatibility (Table. 3). But the goal of efficient cancer chemotherapy has still not been reached by achieving a selective, specific, and least toxic delivery mode, and thus bringing dendrimers to a level generally regarded as safe (GRAS). Hence to fulfill the requirements of this goal some innovative classes of dendrimers, such as liposomal "locked in" dendrimers, came into being.

5. Liposomal "Locked in" Dendrimers

Liposomes, the most extensively studied system for drug delivery, have already been commercialized since formulations of doxorubicin, amphotericin B, and cytarabine are on the market now, and many others are in the clinical phase.[152] In reviewing the history of dendrimers, one encounters the synthesis of many different types of dendrimers, but only a few reports on the synthesis of partial dendrimers bearing lipid character.[153,154] Elsewhere Florence et al.[155] studied the oral uptake of one of the lipid dendrimers. The literature on the use of dendrimers as drug delivery carriers is very large but only a few studies have reported on the interaction of dendrimers and lipids,[156] and the interaction between charged dendrimers and vesicular structures has not been fully investigated. The use of dendrimers as modulators of the release of a drug incorporated into liposomes and the possible alterations of the drug bioavailability seems to be an attractive field for research.

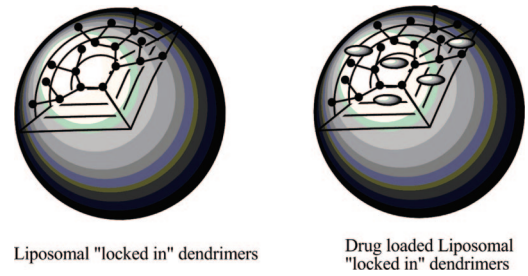

Figure 12. Novel dendro-liposomal concept showing (a) blank "locked in" dendrimer, (b) drug-loaded "locked in" dendrimer.

However, it should be noted that by employing the liposomal concept of drug delivery membrane-permeable drugs (such as doxorubicin) swiftly leak out of the liposomes after dilution or application. Hence, some modes of internal immobilization such as complexation, gelation, precipitation, and membrane binding are also reported elsewhere to give beneficial results.[157] Bosman et al. thoroughly explored the host−guest properties of dendrimers based on physical entrapment, hydrophobic interactions, and ionic interactions.[31] Moreover, dendrimer−phospholipid composites, i.e., dendrosomes, have been reported to be effective in gene delivery.[158]

In the same context the concept of "locked in" dendrimers had been put forward by Purohit et al. in the year 2000.[159] PAMAM dendrimers are only a few nanometers in size,[15] which is almost equivalent in diameter to the thickness of the aqueous space between two liposomal bilayers. Thus, the high positive charges on dendrimers are expected to interact with oppositely charged lipids in liposomes and lead to dendrimer encapsulated inside the aqueous space of liposomes (Figure 12). Purohit et al. prepared the partial dendrimers as reported by Sakthivel et al.[153] The partial dendrimers so prepared are soluble in water and contain three lipidic chains to improve transmembrane transport potential. These are trapped either inside the aqueous phase of the liposomes or in the lipid bilayer and adsorb onto the surface or span the aqueous phase and lipid bilayer. Liposomes were prepared with positive, negative, and neutral charge using the dehydration−rehydration method.[160] The interactions between these liposomes and the partial dendrimers were studied. Taking note of the different interaction efficiencies, it was revealed that there is a degree of partial dendrimer entrapment inside the liposomes (Figure 13). The ζ-potential of the negatively charged liposomes (without additive) was −42 mV, but for the positively charged liposomes it was 26 mV. Consideration of the different interaction efficiencies suggests that there is some partial dendrimer entrapment inside the liposomes. When the cationic partial dendrimers are present, all liposome formulations produced positively charged species with ζ-potential values ranging from 35 to 61 mV. Taking into account the high ζ-potential of dendroliposomes and the negativity of cancerous cells, dendroliposomal constructs can be effectively used for the selective enhancment of the accumulation of loaded anticancer bioactives at a tumorous site.[159]

A milestone for utilization of "lock in" dendrimers concept in drug delivery was first reached by the collaboration of a group of workers from our laboratory and Khopde et al. from the Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.[161] This collaboration advanced the work of Purohit et al.[159] for increasing the entrapment of an acidic anticancer drug, MTX, in liposomes. Previously, it was reported that

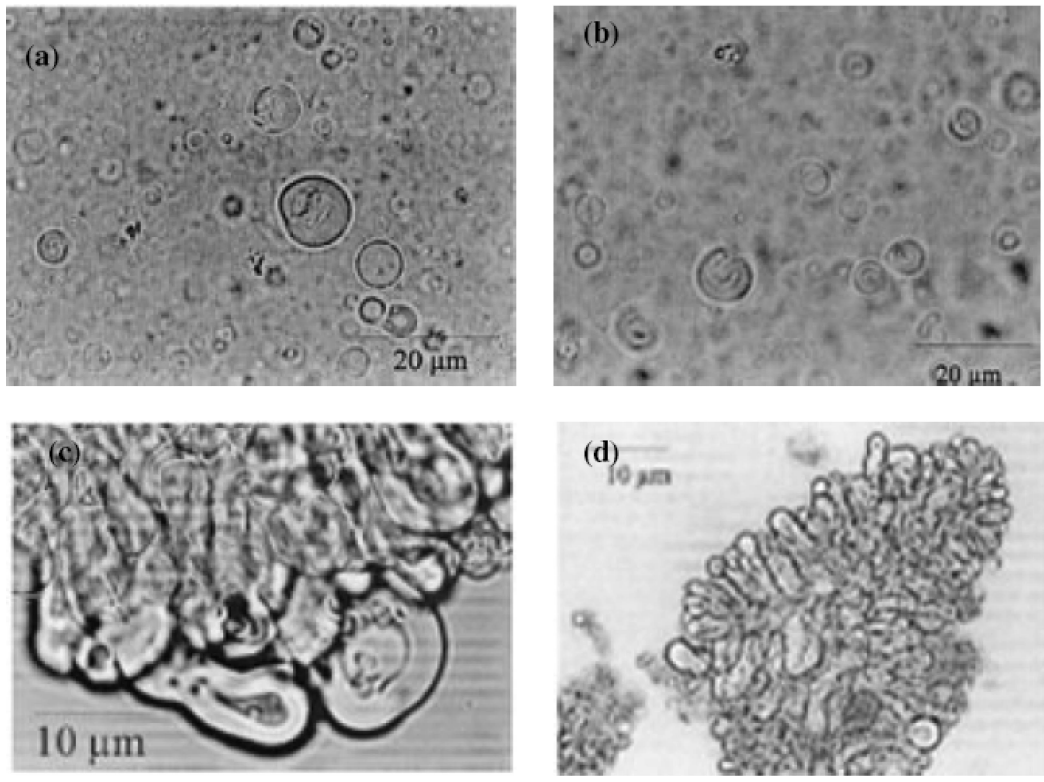

Figure 13. (a) Negatively charged liposomes in the absence of partial dendrimer. (b, c, and d) Negatively charged liposomes in the presence of a 32-amino group partial dendrimer. Adapted with permission from Ref 159. Copyright 2001 International Society for International Journal of Pharmaceutics.

the encapsulation of MTX can be increased by the utilization of the positively charged lipid stearylamine in liposomes because of ion-pair interaction,[162] lipid-conjugation,[163] and proliposome preparation.[164] But on reviewing these data, it can be concluded that the encapsulation efficiency and loading of these methods are unsatisfactory for the delivery of drugs which require relatively high therapeutic doses. Therefore, Khopade, et al. studied the effect of dendrimers on liposome formation and the encapsulation and release of acidic anticancer entities from the conjugates.[161] Encapsulation of MTX molecules in liposomes was observed to increase in the presence of dendrimer. The basicity of the liposome interior caused by a dendrimer creates a pH gradient that may be the reason for the increased influx of MTX. The encapsulated dendrimer seems to serve as a sink in the uptake of MTX molecules. However, within 8 h the release rate of MTX was lowered from 65% to 25% for dendrimeric liposomal formulations. The entrapment of drug was proportional to the generation of dendrimer. The liposomes containing 4.0G dendrimers encapsulated the drug approximately 2-fold and 4-fold more than did the liposomes containing the 3.0 and 2.0G dendrimer, respectively. It was further reported that these systems were birefringent under polarized light (Figure 14), and that their architecture resembles a wormlike gel structure which tends to elongate and break or fuse on the application of shear on a glass slide.

For all anticancer drugs, the discharge rate is an important constraint, and a slow release is essential in diminishing unwanted side effects and improving the therapeutic index, as was explained by Horovic et al.[165] Later Papagiannaros et al.[166] developed a liposomal formulation incorporating a doxorubicin PAMAM complex, which ensured the controlled release of doxorubicin from the liposomes and effectively reduced side effects and improved the therapeutic index. These formulations exhibited physicochemical characteristics and release properties suitable for avoiding the fast release of cytotoxic drugs from conventional liposomes.

Aristarchos and co-workers cleverly reviewed aspects of liposome formulation in relation to anticancer activity. Engelman et al. previously reported hexadecylphosphocholine to be an antitumor ether lipid, and it is generally used in several European countries to treat metastases of breast cancer.[167] They reported that the hydrophobic forces between the lipid and dendrimer dominate in the formation of such architectures. The same conclusion was arrived at using "dendrons" by Purohit et al.[159] The doxorubicin—PAMAM complex attached to liposomes formulated from hexadecylphosphocholine/egg phosphatidylcholine/stearylamine in 10:10:0.1 molar ratio showed better in vitro outcomes.

Despite this, the scientific community is well aware of the existing stability problem associated with liposomes. Studies are continuing toward finding newer reliable delivery devices with maximal stability. One possible way was offered, using doxorubicin as a model construct, by a research associate from our laboratory. We employed 4.0G PAMAM dendrimers to develop dendrimer/poly(styrenesulfonate) (PSS) microcapsules following a layer-by-layer deposition protocol of both constituents around a removable melamine formaldehyde colloidal core. These PAMAM/PSS constructs allowed the selective encapsulation of drug into the dendrimers, which in turn enhanced the localization within the shell of the capsule.[74] Such tactics may offer even more stability than the liposomal "locked in" constructs; it only remains for the scientific community to implement them.

In conclusion, these "locked in" dendrimers could be regarded as an efficient class of dendrimer-based modulatory liposomal controlled release systems (MLCRS) with a great potential for carrying a high drug load and its subsequent

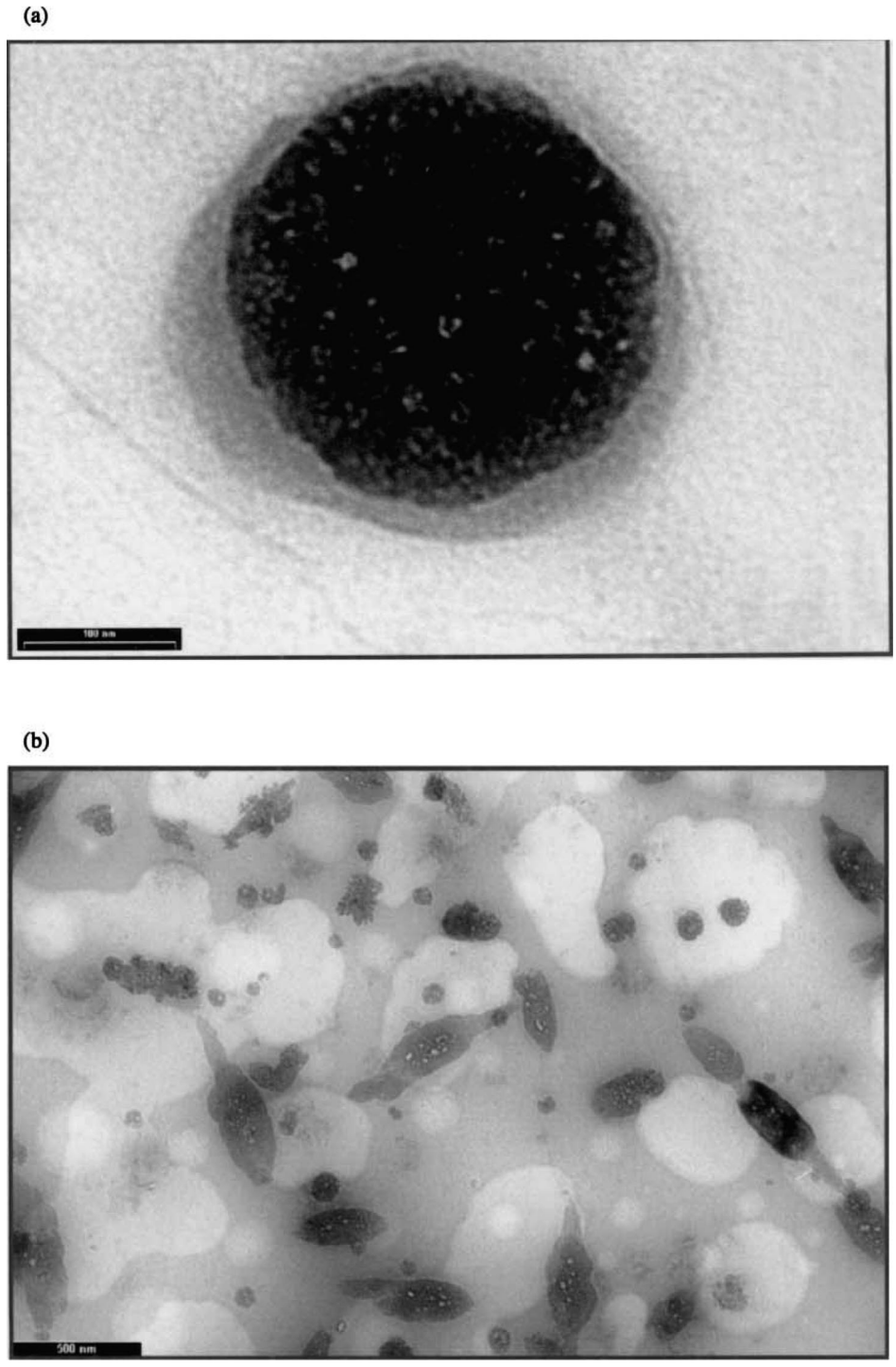

Figure 14. (a) TEM image of dendrimer containing liposomal formulation; (b) single lipid particle showing encapsulated dendrimer grains. Adapted with permission from Ref 161. Copyright 2002 International Society for International Journal of Pharmaceutics.

modified release so that they are capable of reducing the side effects as well as augmenting the therapeutic index profile of the loaded bioactives. But they are still not completely characterized and therefore require further detailed exploration.

6. Site Specific Dendritic Scaffolds

"Targeting drugs directly to cancer cells reduces the amount that gets to normal cells, increases the drug's anticancer effect, and reduces its toxicity" says one of the principal investigators, James R. Baker. Tumor cells overexpress many receptors and biomarkers, and these can be used as targets to deliver cytotoxic agents into tumors. In general, a tumor-targeting drug delivery device consists of a tumor recognition moiety connected directly or through a suitable linker to a cytotoxic drug to form a conjugate. This demands that the linker must be stable in circulation, but the conjugate should be readily cleaved to regenerate the active cytotoxic agent upon internalization into the cancer cell.

The past few decades in particular have witnessed significant progress in the area of controlled and targeted delivery. The notion of site-specific controlled and targeted drug delivery via different engineered particles[117,118] not only

promises to reduce toxicity but also expands the therapeutic window of cancer drugs, thereby giving hope for turning cancer into a chronic though manageable disorder. This endeavor in particular may necessarily fulfill the potential of targeting, which is now expanded to the concept of "complete targeting" by the advent of more capable scaffolds called dendrimers.[41]

The low polydispersity index, appreciable water solubility, lack of immunogenicity, and in particular the presence of a great number of modifiable peripheral amine groups make dendrimers well suited to serve as a platform for the development of an effective tumor targeting drug delivery device.[76,172,173] It is likely that the majority of the other delivery devices employed for the same purpose would not readily gain access to tumors from the vasculature because their larger diameters (between 40 and100 nm) are too big to cross vascular pores.[174] Dendrimers are of the same size as small serum proteins and thus can enter through pores in the vasculature and infuse tumor cells directly.[175] Another advantage is that this type of drug-delivery may overcome the effect of the multidrug resistance channel.[176] The following section of this review describes recent advances in tumor-targeted dendrimer conjugates with stress on the areas of folic acid, glycodendrimer, RGD, and monoclonal antibody mediated tumor targeting.

6.1. Folic Acid Guided Nanocarriers

Folate conjugation has a very long history in cancer chemotherapy. Folic acid is a B-vitamin and is indispensible in the formation of new cells since it is one of the pivotal constituents utilized by mammalian cells for the biosynthesis of methionine, serine, deoxythymidylic acid, and purines. Leuchtenberger et al. first documented the connection between tumors and folic acid in 1944,[177] when it was observed that an extract from *L. Casei* factor inhibited the enlargement of murine cancer. Later, it was found that the active principle in this extract was a peptidic conjugate of folic acid with two glutamic acid residues.[178] Subsequent studies revealed that the high affinity folate receptor (KD < 1 nM)[179] is selectively overexpressed in many tumors of epithelial origin.[180] Some of these tumors include ovarian,[178,180−184] lung and colon,[185] kidney, breast, choriocarcinomas, choroid plexus brain tumors,[175] and also childhood ependymomas.[186]

Similarly there are a number of studies reporting in detail the differential expression of the folate receptor in diseased tissue.[187,188] Weitman et al. performed an experiment to confirm folic acid as an efficient anchor in tumor targeting. The radiolabeling study showed that tritium-labeled folic acid binds specifically to tumor cells 20-fold more than to normal body cells.[185] Since then, folic acid has been continually used as a hook to anchor the folate receptors overexpressed on the tumors' balcony.

Recently, this idea has been employed for camptothecin (CPT), by using polyethylene glycol to serve as a spacer/scaffold between CPT and folic acid.[189] Still, the reports stressing direct folate conjugation of anticancer drugs are fewer and are not particularly convincing compared to the concept of folate anchored delivery devices. Likewise there are a number of papers describing how the direct conjugation of folate to the bioactive molecule can lead to the defeat of targeting or alteration of the bioactivity of the conjugate. Such reports paved the way for a second concept of the folate-anchored approach to be explored.[5,191−194] This second approach focused mainly on the anticancer bioactive or contrast agent bearing macromolecular conjugates: polymeric micelles,[195] liposomes,[196−198] synthetic polymers,[199] nanoparticles,[200] proteins,[201] protein toxins,[202] and MRI agents.[203] However, since the advent of dendrimers with the notable assets to their credit, they have become the vehicle of choice in folate-guided attacks on cancer. This attack delivers such conjugates to cells through receptor-mediated endocytosis.[191,204,205] Utilization of folic acid for the active targeting of dendrimers is to a great extent inspired by the pathbreaking ideas of Esfand and Tomalia,[14] and Kono et al.[206]

Previously, several research groups have already demonstrated the utility of the internal void volumes of dendrimers in encapsulating hydrophobic agents.[112,207−209] Among the vast dendrimeric family, the PAMAM dendrimers have been considered extensively for varying medical applications.[8,210] It is clear from the studies of Kohle and co-workers[70−72] and Zhou et al.[122] that in addition to the complexation of hydrophobic drugs within the hydrophobic interior, dendrimers are also capable of covalently tying the drugs onto the multivalent surface.

Manipulating these concepts along with the Wiener et al.[172] folate targeting approach, Patri et al.[211] compared the efficacy of 5.0G PAMAM dendrimer conjugates in targeted delivery. They compared the release kinetics of drugs from covalently conjugated and drug inclusion complexes. The dendrimer-drug inclusion of MTX was found to enhance its water-solubility, but the association was found to be short-lived. Although this complex was stable in water with minimal release (less than 5%) of free MTX when dialyzed against water, more than 70% of the incorporated MTX was released within 2.5 h in PBS. In contrast, MTX covalently conjugated to dendrimer is released neither in water nor in PBS. Cytotoxicity studies showed that the ester-linked covalent conjugate required about 10-fold higher concentration to have the same effect as the inclusion complex. This might be due to a slower release of conjugated MTX from the conjugate after receptor-mediated internalization[113,114] (Figure 15).

The antineoplastic drug MTX is a folic acid antagonist with several hundred fold lower affinity for folate receptors than folic acid. Kono et al. synthesized polyether dendritic compounds bearing folate molecules on their surface as a model drug carrier construct. Hydrazide groups were created by the reaction of the dendritic surface with hydrazine, and then conjugation of the folate residues to the hydrazide chains by either direct condensation with folic acid or by reaction with an active ester derivative of folic acid. They were successful in attaching an average of 12.6 folate residues to each dendrimer. In addition, conjugation of MTX to the dendrimers was also explored and its coupling with the dendritic surface occurred via the same chemistry as expected for folic acid. An average of 4.7 MTX molecules were conjugated to the dendritic surface. The conjugates were reported to be soluble in PBS maintained at physiological pH. Further, on the basis of the structure of the conjugate, it was postulated that it could be successfully employed to entrap hydrophobic drugs.[206] This study was the first to produce the drug-conjugated folate-dendrimers in a well-matched manner and thus lay the foundation for achieving the vision of complete targeting.

Ideal antitumor therapeutics must have multiple functions, such as targeting a tumor, imaging the presence of the tumor, and delivering a therapy to tumor cells.[212] Probably with

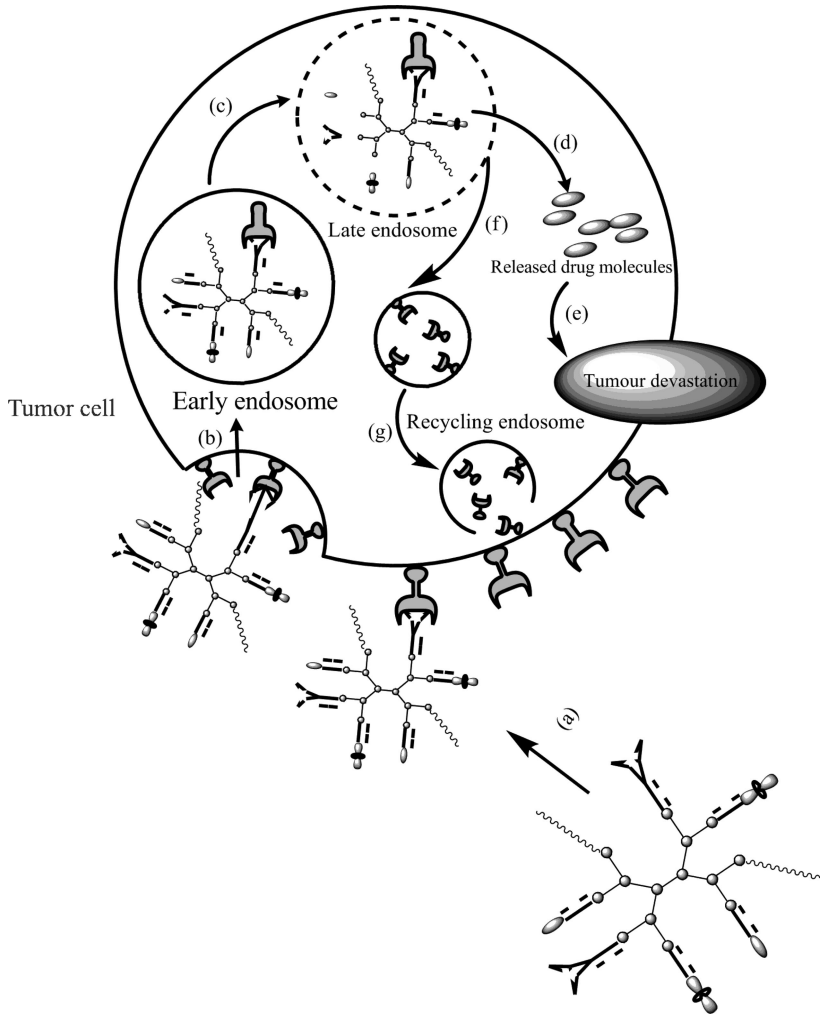

Figure 15. Schematic diagram showing folate receptor-mediated delivery of folate-anchored dendrimers to the cancer cells: (a) association of folate-anchored dendimers with folate receptors (KD approx 50 pM); (b) folate receptor associated conjugate will traffic into the cells by receptor-mediated endocytosis; (c) endosomal disruption; (d) release of endosomal content into cell cytoplasm; (e) therapeutic agent related bio events followed by tumor devastation; (f) the unligated folate receptor recycling toward the cell surface; (g) membrane fussion followed by restoration of surface receptor.

this in mind, Quintana et al. presented a hybrid concept with the intent of developing a nanoscale drug delivery device that would allow targeted intracellular delivery along with an imaging capability for tracking uptake of the material. The device was based on an ethylenediamine core 5.0G PAMAM dendrimer. Targeting, imaging, and intracellular drug delivery capabilities were to be provided by attaching folic acid, fluorescein, and MTX, respectively, to the same system. Three surface modified nanodevices were synthesized by capping the dendrimers with acetamide, hydroxy, and 2,3-dihydroxypropyl functionalities. MTX was conjugated either through a nonbiodegradable amide linkage or through an ester linkage that would hydrolyze at the low pH found in the endosome. The optimal dendrimer surface modification was determined by molecular modeling, which suggested that all folic acid targeting moieties in the acetamide-capped dendrimers appeared to extend away from the surface of dendrimer, thus optimizing the likelihood of receptor interaction. The highest apparent affinity was observed with the acetamide-capped nanodevices, which resulted in a 20-fold increase in cell fluorescence, and the experimental targeting data in KB cells confirmed the modeling predictions.[213]

Recently Thomas et al. studied the cellular uptake and cytotoxicity of an engineered multifunctional dendritic nanodevice containing folic acid (FA), fluorescein (FL), and MTX. The conjugation of the former two moieties to 5.0G PAMAM dendrimer was carried through a thiourea and amide linkage, while the drug was conjugated through an ester linkage to generate the trifunctional dendritic device (PAMAM−FI−FA−MTX). The targeted dendrimer conjugates were effective in inhibiting cell growth in KB cells as compared to their nontargeted counterparts, thus showing the potential of folate-targeted conjugates for targeting and growth suppression of tumor cells that overexpress FA-receptors. But, compared to free MTX, the trifunctional conjugate PAMAM−FI−FA−MTX showed a reduced antiproliferative effect caused by the slow hydrolysis and release from the PAMAM−FI−FA−MTX conjugate of the folic acid, which then acted as a rescuing agent to reverse the cytostatic effect of the antifolate MTX.[214] Calvert previously stated that the potency of free MTX to induce cytotoxicity is caused by its polyglutamation activity, as a result of which entrapment and accumulation of the drug occurs inside the cell.[215] The physiological polyglutamation of MTX takes place at its β-carboxyl group. Since the chemical conjugation of MTX to the dendrimer is through the β-carboxyl group of the MTX, it follows that such polyglutamation in the conjugate cannot occur. Anyway, it was suggested that the conjugation of the MTX to the dendrimer delayed its exit from the cell through the exit

pump. This was further supported by confocal microscopy data, which showed the retention of FA-conjugated dendrimers in KB cells which had been preincubated with the conjugate for 24 h followed by incubation in a dendrimer-free medium for 4 days.[214]

Researchers at the University of Michigan recently conjugated acetylated dendrimers to FA and then to either MTX or tritium and either fluorescein or 6-carboxytetramethyl rhodamine. As compared to the free drug, intravenous injections of the conjugate was 10 times more effective in delaying growth of human tumors in immunodeficient mice bearing human KB tumors and were also capable of extending the life of the mice under study. In contrast to nontargeted polymer, folate-conjugated nanoparticles concentrated in the tumor and liver tissue after 4 days of administration, suggesting specific uptake against a concentration gradient of dendrimer in these tissues. This drug conjugate showed internalization into tumor cells as was further confirmed by confocal microscopy. There was no gross acute or chronic toxicity, suggesting a much higher dose could be used than with the free drug.[216]

The purity of synthesized conjugates is of great concern. Therefore, Baker et al. performed extensive studies on folate anchored bi- and tri-PAMAM-nanocarriers. Such studies comprised a wide array of branches, with a recently reported one being the HPLC analysis of folate−PAMAM dendrimer-based multifunctional devices. Methods have been developed for the detection and separation of surface-functionalized dendrimer conjugates of small molecules (FITC, FA, MTX) using a common gradient. Such techniques can be used to optimize the physicochemical properties of the conjugates to improve drug targeting to cancer cells.[217] The molecular distribution of PAMAM conjugates largely depends on the homogeneity of starting materials, the synthetic approaches, and the final functionalization steps. Very recently, size exclusion chromatography and capillary electrophoresis were used for analyses of the molecular heterogeneity in folate−PAMAM multifunctional nanodevices (5.0G PAMAM −FA−MTX). Such applied analysis of mono- and multifunctional PAMAM-based nanodevices provides a superior tool for the evaluation of molecular heterogeneity in complex nanodevices.[218]

6.1.2. DNA-Assembled Dendrimer−Folate Conjugates

Parallel studies also indicated that the conjugation of multiple numbers of molecules on to the surface of single dendrimer may give synthetic problems, viz., hampered water solubility and poor yield, probably due to steric hindrances and the hydrophobicity of attached functionalities. Often, such techniques of achieving a cancer cure demand bioactive alterations in various specific tumor types. Baker and coworkers have proposed that the self-assembly of PAMAM dendrimers using complementary single-stranded oligonucleotides could be a versatile alternative approach for constructing a common combinatorial anticancer therapeutics.[213,222]

Hence, to fulfill this goal for common combinatorial anticancer therapeutics, Choi et al. constructed a biofunctional dendrimer conjugate with fluorescein isothiocynate [FITC] and FA moieties and then linked them together using cDNA oligonucleotides to generate clustered molecules that target cancer cells that overexpress the high-affinity folate receptor (Figure 1j). Amine-terminated 5.0G PAMAM dendrimers are first partially acetylated and then conjugated with FITC or folic acid, followed by the covalent anchoring of complementary, 5′-phosphate-modified 34-base-long oligonucleotides. Hybridization of these oligonucleotide conjugates lead to the self-assembly of the FITC and folic acid conjugated dendrimers. In vitro studies of the DNA-linked dendrimer clusters confirmed specific binding to KB cells expressing folate receptor, and internalization of the dendrimer cluster was confirmed by confocal microscopy. This established the ability to design and fabricate supramolecular arrays of dendrimers using oligonucleotide bridges, which can be followed further for development of DNA-linked dendrimer clusters in imaging and therapeutics.[219]

Choi and Baker also reported similar studies employing cDNA oligonucleotides to generate clustered biofunctional moieties FITC and FA that targets cancers overexpressing high affinity folate receptors. This DNA-linked dendrimer nanocluster podium is only a few nanometers in diameter and is now considered a milestone in the delivery of genetic resources and imaging agents, and also offers combinatorial therapeutics for cancer cells.[220] Recently, Iwamura at Toho University in Japan has also reported a similar construct employing PAMAM dendrimers.[221]

6.1.3. Multimodality Dendrimer-Based Diagnostic Agents

The need to develop target-specific contrast agents to aid in designing tumor imaging agents has always been very important. Macromolecular contrast agents have been reported to play an active role in imaging to enhance tumor selectivity, either by active or passive targeting. Passive targeting of such agents involves their nonselective accumulation in tumors as a result of enhanced endothelial permeability.[223] The active targeting approach may further help in selectively targeting these imaging agents to the tumor.

Hence, as a move toward increasing the number of gadolinium atoms per antibody, polymeric gadolinium complexes were utilized. In this approach the amino groups of poly-L-lysine were conjugated to approximately 65 Gd−DTPA molecules and then to an antibody with high specificity for colon carcinomas. Though this succeeded in achieving enough Gd accumulation in the tumor and signaled enhancement, it raised concerns relating to high production costs and potential immunologicity.[224] Particulate contrast agents based on magnetite, monocrystalline iron oxide nanoparticles, and ultrasmall superparamagnetic particles of iron oxide have also been reported. In addition, active tumor targeting has been achieved with antibody-conjugated paramagnetic liposomes (ACPL). Likewise, several systems have been tried, but none have reported complete success, in achieving the goal.[225−233]

However, the responsibility was shifted to the shoulders of dendrimers, a very flexible instrument with respect to surface and conjugation chemistry, to accomplish the goal. Dendrimers solved the problem of the size and the availability of surface groups for conjugation to an imaging label, since the number of surface functional groups grows geometrically as the diameter of dendrimers grows linearly, which makes the molecules appealing for biomedical applications.[234,235] Moreover, their larger size offers longer imaging times and allows elevated relaxation rates because of their longer rotational-correlation times.[125,236,237] For such applications of multivalent scaffolds, the wedge-shaped or spherical ones such as PAMAM dendrimers are the most frequently used.[238]

Imaging of the sentinel node is frequently performed before surgery of breast and melanoma. Existing methods rely on radio-lymphoscintigraphy; MR lymphangiography offers the benefits of better spatial resolution without ionizing radiation. However, the best nanoparticle size for imaging the sentinel nodes remains unclear. Recently, to solve this problem, gadolinium-labeled contrast agents (between 1 and 12 nm) were evaluated to determine which size provides the most rapid and concentrated delivery of contrast agent to the lymph nodes in a mouse model of lymphatic metastases. Different generations of PAMAM (2.0, 4.0, 6.0, 8.0G) and Gd-agents, as well as Gadomer-17 and Gd−DTPA were compared. Among these agents, the 6.0G PAMAM Gd showed the lymphatic and lymph nodes with the highest concentrations 24−36 min after injection, thus offering a basis for utilization of the device in targeted therapy of sentinel nodes.[239] Recently, Talanov et al. have demonstrated the potential of a PAMAM dendrimer-based nanoprobe for dual magnetic resonance and fluorescence imaging for efficient visualization of sentinel lymph nodes in mice.[28]

Wiener and co-workers have produced polymeric dendrimer chelates by coupling 2-(4-isothiocyanatobenzyl)-6-methyldiethylene triamine pentaacetic acid (TU−DTPA) and 2-(4′-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid to ammonia core PAMAM dendrimers.[125,240] When such chelates are complexed to Gadolinium they yield an effective magnetic resonance imaging contrast agent. The group has reported that the 4.0G PAMAM-contrast agent conjugate might significantly alter the relaxation rate of the cells.[172] In 1994, Wiener et al.[125] reported that the relaxivities of the dendrimer-based agents depend on the generation of dendrimer used, and the variance is 2−6 times that of low molecular weight contrast agents for generation two through six of the PAMAM−TU−DTPA derivative. However, such conjugates offered a great deal of nonspecificity in delivery.

To address the serious need for target-specific MRI contrast agents with high relaxivity, in 1997 Wiener et al. developed for the very first time a new method for delivering contrast agents to tumor cells that overexpress the folate receptor. The authors attached FA to a 4.0G ammonia core PAMAM dendrimer. These folate-anchored dendrimers were then reacted with TU−DTPA to form a polymeric chelate, folate−PAMAM−TU−DTPA. For fluorescence studies, these dendrimers were reacted with FITC and carboxytetramethyl rhodamine succinimidyl ester. Tumor cells expressing high affinity folate receptors (hFR) showed a rapid rise of fluorescence to 350% followed by a slow increase up to 650%, suggesting two phases of uptake: a rapid phase associated with initial binding of the conjugate to cell surface receptors and a slower phase consistent with internalization of receptor−conjugate complex followed by dissociation and receptor recycling. The group also reported that the Gd complex of folate−PAMAM−TU−DTPA resulted in a specific increase of ∼109% in the longitudinal relaxation rate, thus confirming the previous findings of the same group.[125] Moreover, when the cells were treated with both free folic acid and folate−PAMAM−TU−DTPA, there was only a 20% increase in the relaxation rate, thus projecting the role of folate in the targeting.[172]

Later Konda et al. studied the in vivo performance of a similar dendro-folate-contrast agent conjugate and reported that the folate−dendrimer conjugate shows noteworthy accumulation in xenografted ovarian tumors expressing the folate receptor resulting in a significant signal enhancement compared to a nonspecific extra-cellular contrast agent (Gd−HP−DO3A). This group also measured the relaxivities of the folate conjugated dendrimeric conjugate and reported that the relaxivity was 8-fold greater than that of Gd−HP−DO3A. The percentage contrast enhancement after 24 h of injection was determined using the proposed equation and was found to be increased by 33%.[241]

In another follow through study, Wiener and co-workers specifically targeted the ovarian tumor xenografts for imaging by using the same conjugate. The radiolabeled folate−dendrimer chelates selectively bind to these high affinity folate receptors, resulting in an increase of over 2700% in binding compared to untreated cells. Following the in vivo administration of folate−dendrimer chelate in ovarian tumor xenografts resulted in a 33% contrast enhancement.[242]

In addition to this work Konda et al. also reported the biodistribution of Gd−folate−dendrimer chelate in hFR positive and negative ovarian tumor xenografts. The hFR-positive tumors accumulated a significantly higher dose of around 3.6% of the injected dose (%ID)/g after 24 h, while only the background amount was found in hFR-negative tumors. Similar trends were observed when %ID/organ data was compared. Low levels of agents were found in blood (<1.9% ID/g), with kidney showing the highest level.[243] Mantovani and Miotti reported the presence of folate receptors on the proximal convoluted tubule of kidney,[244] and this may be one of the reasons for this accumulation of Gd−folate−dendrimer in both kidneys.[243]

In the past, Li and co-workers had developed polymer−drug conjugates using linear poly(L-glutamic acid) [PGA] as the drug carrier for paclitaxel. The resulting PGA−paclitaxel conjugate exhibited enhanced antitumor activity as compared to that of the parent drug, probably due to the EPR effect of macromolecules.[245,246] Tansey et al. have used PGA in applying the active targeting concept in addition to the EPR effect. They described the synthesis and characterization of a novel folate nanoconjugate containing multiple branched PGA chains centered on a PAMAM dendrimer. These polymers were degradable in the presence of the lysosomal enzyme cathepsin B, albeit quite a bit more slowly than was linear PGA. Furthermore, the near-infrared dye indocyanine green, a model diagnostic agent, was conjugated to the terminus of the scaffold. Binding of a novel PAMAM−PGA−folate conjugate to tumor cells was studied using a human nasopharyngeal epidermal carcinoma cell line, KB, that overexpresses folate receptors and a human breast carcinoma cell line SK-Br3 that has no detectable folate receptors.[247] The binding results for the conjugate were similar to those reported earlier.[189,241,242] Moreover, these structural frameworks are biodegradable and water-soluble, which makes them attractive for consideration as drug delivery devices for requiste purpose.[247]

Uppuluri et al. have reported on the synthesis of tectodendrimers. Such clusters have been prepared with fluorescein in the core reagent for detection and folate as the targeting moiety. Such conjugates solved the solubility problems encountered in previous studies with aromatic FITC moieties on dendrimeric surfaces (Figure 1g). This conjugate was suggested to be far superior to those dendrimeric conjugates containing both FITC and folic acid attached to the surface.[248] Moreover, there have been a few reports on the preparation of water-soluble dendritic polymers with

heterodifunctional groups that can be used for the attachment of both targeting moieties and diagnostic or therapeutic agents.[249]

Such results from many different laboratories and over a broad spectrum of examples suggest that folate-fortified dendrimers will soon find a vital niche in cancer treatment methodology (Tables 4−5). Also, glycodendrimers, RGD coupled dendrimers, and antibody/receptor guided dendrimers all in conjunction with folate−guided targeting may finally lead to a safe, effective, and reliable cancer cure.

6.2. Glycodendrimers in Cancer Targeting

The special biology of carbohydrate receptors requires these carbohydrates to be clustered so as to attain biologically meaningful affinities for the receptors. Because of rapid advances in the area of carbohydrate receptors and their ligands, promising areas of applications for the carbohydrate clusters have appeared. These include an array of medical wonders, the chief among them being the treatment of cancers (including breast cancer, renal cancer, and melanoma), the eradication of cancer metastases, the management of metabolic disorders involving carbohydrates, and protection against infection by flu and pathogenic strains of *Escherichia coli* and *Clostridium difficile*.[250] Carbohydrate clustering has been used in many ways, including attachment to natural products and synthetic polymers, the preparation of synthetic glycopeptides, and simple oligomerization through short organic linkers.[251] The change in the repertoire of surface carbohydrates accompanying malignant transformation has been an area of extensive investigation and the basis for strategies aimed at recruitment of the immune reaction against the tumors.[252,253]

Glycodendrimers are constructs having several surface carbohydrate residues accessible for multiple binding interactions. To find such constructs, several T-antigen containing glycodendrimer clusters were synthesized and evaluated for their relative binding properties.[254−256] Even though glycodendrimers may be less efficient than glycopolymers and neoglycoproteins in inhibition experiments, they have been of interest because of their lack of immunogenicity and their better defined molecular architecture. Furthermore, the synthetic tactics used in forming glycodendrimers allow the fabrication of more firm, that is, conformationally restricted, molecules. Since glycodendrimers are chemically and geometrically well-defined monodisperse macromolecules, they are suitable as tools for medicinal and phamaceutical purposes.

The potential applications of PAMAM dendrimers to biological systems is broadened by their combination with different carbohydrate moieties.[257,258]

Chemically and geometrically well-defined T-Ag glyco-PAMAM dendrimers with valencies of 4, 8, 16, and 32 were synthesized via proficient amide bond formation. Successive bioassays showed strong protein binding properties, thus demonstrating an excellent cluster effect and establishing these as strong candidates for biological and immunochemical applications such as the inhibition of cancer cell metastasis.[259]

A literature review revealed that sulfation of the oligosaccharides resulted in an increase of their antitumor and anti-HIV activities.[260−265] Du et al. reported the synthesis of sulfated glucan derivatives, in which the sulfated hexa-b-D-glucoside also was a potent antitumor agent based on Sarcoma-180 model studies in mice.[266]

The synthesis of a series of carbohydrate-coated dendrimers was reported a few years ago.[267] These compounds are members of a rapidly expanding family of neoglycoconjugates, the so-called glycodendrimers,[268−271] which have become the subject of extensive investigation[272] and are emerging as potent ligands for carbohydrate-binding proteins.[273−275]

The PAMAM and polylysine−based glycodendrimers have been successfully evaluated functionally in a model of the rat-natural killer receptor-protein 1 (NKR-P1) receptor system for tumor eradication using both rat colorectal carcinoma and B16 murine melanoma models. Survival was prolonged from an average of 27 days in the control group and groups receiving various other treatments, to 42 days in the treated group.[276]Pospisil et al. reported that a single dose of the glycocluster could be a substitute for multiple injections of the neoglycolipid-coated liposomes. This specific effect was found to be mediated by a specific triggering of cellular immunity in NKR-P1 cells, which led to the subsequent elevation of the CD4 lymphocyte subset, and hence to a permanent immune response against the tumor.[277,278]

Roy et al. had synthesized novel glycodendrimers based on an *N*,*N*′-bis(acrylamido)acetic acid core. The breast cancer-associated T-antigen carbohydrate marker was then conjugated by thiolated T-antigen to the *N*-acrylamido dendritic cores in the first case, and by amide bond formation between an acid derivative of the T-antigen and the polyamino dendrimers in the second case. The multivalent conjugate showed improved inhibitory effects compared to monovalent antigen.[279] Later this same group studied a similar antigen, [β-D-Gal-(1-3)-α-D-GalNAc], with different scaffolds including PAMAM, PPI, *N*,*N*′-bis(acrylamido)acetic acid, and finally hyperbranched L-lysine, for constructing glycodendrimers. A few glycodendrimers were also linked to fluorescein and biotin probes to track T-Ag binding.[280] Veprek et al. have investigated the molecular dynamics of such glycodendrimers to examine structural differences taking place during synthesis.[281] Later, Roy and Kim prepared building blocks containing the bipyridyl group in a convergent manner using 2,2′-bipyridine-4,4′-dicarboxylic acid chloride and the amine-functionalized sugar derivatives. They evaluated the relative inhibitory potencies of these glycodendrimers against monomeric allyl α-GalNAc. The di- and tetravalent bipyridyl clusters showed an 87-fold increase in inhibitory properties when compared to the monomeric counterpart.[282]

From the earlier reports of Pospisil and co-workers, it is now quite clear that such changes on cancer cells, because of their aberrant glycosylation, offer an excellent potential as targets for immune recognition through lectin-like receptors on immune cells. These cells include natural killer CD8+ and CD4+ lymphocytes together with cytokines necessary for important functions in antitumor immunity.[277,278] Vannucci et al. later again manipulated glycodendrimers as an approach to anticancer immune modulation through carbohydrate-mediated immune recognition. This group employed octavalent PAMAM dendrimers functionalized with *N*-acetylglucosamine residues (PAMAM-GlcNAc8), which had in vitro high affinity for the recombinant lymphocyte receptor NKR-P1. To follow the fate of the conjugate, a fluorescent marker was conjugated to the tetrabranched semicomponent of the dendrimer. Tumor development and immunity were evaluated in C57BL/6 mice. Animals were inoculated with B16F10 melanoma cells and underwent different protocols of PAMAM-GlcNAc8 administration. They reported that the

Table 4. Folate-Guided Dendrimers in Tumor Targeting

type of dendrimer	imaging agent	drug studied	mode of drug attachment	objective of study	reference
polyether dendritic		MTX	covalent conjugation	to investigate folate and MTX conjugation to dendrimers.	Kono et al.[206]
PAMAM		MTX	both inclusion as well as covalent conjugation	to compare of release kinetics of covalently conjugated and inclusion complex.	Patri et al.[211]
PAMAM	FL	MTX	covalent conjugation	to develop targeted nanodevice with imaging capability for tracking cellular uptake.	Thomas et al.[214]
surface-modified PAMAM	FL	MTX	covalent conjugation	to develop targeted nanodevice conjugated to drug and imagingagent and bearing imaging capability for tracking uptake. Molecular modeling to determine optimal surface modification.	Quintana et al.[213]
PAMAM	FL or 6-CTMR	MTX	covalent conjugation	to assess proficiency of conjugate in delaying enlargement of tumors and toxicity monitoring in immunodeficient mice bearinghuman KB tumors.	Kukowska-Latallo et al.[216]
PAMAM	FITC	MTX	covalent conjugation	provides yardstick data, which can be used to optimize the physicochemical properties of the conjugates to improve drug targeting to cancer cells.	Islam et al.[217]
PAMAM		MTX	covalent conjugation	provides a commanding tool for the evaluation of molecular heterogeneity in folate-targeted nanodevices.	Shi et al.[218]
complementary DNA oligonucleotides assembled PAMAM.	FITC			suggests strategy to overcome steric hindrances and hydrophobicity of attached functionalities.	Choi et al.[219]
complementary DNA oligonucleotides assembled PAMAM cluster.	FITC			to develop DNA-linked dendrimer nanocluster podiums which are only few nanometers in diameter for offering combinatorial therapeutics for cancer cells	Choi and Baker.[220]
DNA linked (FA-PAMAM and FITC-PAMAM)	FITC			to develop biocompatible and effective nanodevice for tumor targeting.	Iwamura[221]

Table 5. Folate-Anchored Dendrimers in Tumor Diagnosis

name of dendrimer	imaging agent studied	objective of study	reference
PAMAM	Gd−DTPA	to develop finest nanoparticles for imaging the sentinel nodes.	Kobayashi et al.[239]
PAMAM	2-(4-isothiocyanatobenzyl)-6-methyldiethylene triamine pentaacetic acid and 2-(4′-isothiocyanatobenzyl)-1,4,7, 10-tetraazacyclododecane-*N,N′,N″,N‴* -tetra acetic acid.	to fabricate effective MRI contrast agent.	Wiener and co-workers.[125,240]
folate−PAMAM	TU−DTPA	to address the need for target-specific MRI contrast agents with high relaxivities.	Wiener et al.[172]
folate−PAMAM	TU−DTPA	to perform the in vivo performance of dendro-folate-contrast agent.	Konda et al.[241]
tecto dendrimers	FITC	to overcome the solubility problem encountered in previous studies with aromatic FITC moieties on dendrimer surface.	Uppuluri et al.[248]
folate−PAMAM	TU−DTPA	to develop targeted dendrimeric MRI agent possessing high molecular relaxivities and effective tumor imaging ability	Konda et al.[242]
folate−PAMAM	TU−DTPA	to study the biodistribution of Gd−folate−dendrimer chelate in hFR positive and negative ovarian tumor xenografts.	Konda et al.[243]
folate−PAMAM−PGA	near infrared dye indocyanine green, a model diagnostic agent	to study the binding outcomes of novel PAMAM−PGA−folate conjugate to tumor cells.	Tansey et al.[247]

survival and tumor growth inhibition effects behaved in a dose-dependent manner by the intraperitoneal route. Besides the increase of CD69+ cells in the spleen and their appearance inside the tumors, early release of IL-1β and subsequent production of INF-γ and IL-2 concomitant to an increment of CD4+ cells were also observed. Cytotoxicity assays, performed ex vivo, showed an enhanced NK cell activity proportional to the percentage of activated NK cells. This clearly suggests that the multivalent neo-glycoconjugates can stimulate an antitumor immune response in tumors.[283]

Shaunak et al. used anionic 3.5G PAMAM dendrimers to make water-soluble conjugates of D(+)-glucosamine and D(+)-glucosamine 6-sulfate with immuno-modulatory and antiangiogenic properties, respectively. Dendrimer glucosamine 6-sulfate blocked fibroblast growth factor-2 mediated endothelial cell proliferation and neo-angiogenesis in human Matrigel and placental angiogenesis assays. Further, their report suggests that synthetically engineered macromolecules such as the dendrimers they describe can be tailored to have defined immuno-modulatory as well as antiangiogenic properties.[271]

6.3. RGD-Coupled Dendrimers in Antiangiogenic Therapy

Antiangiogenic therapy is yet another approach for dealing with cancer that involves the prevention of neovascularization by inhibiting proliferation, migration and differentiation of endothelial cells. The identification of markers that can distinguish newly formed capillaries from their mature counterparts paved the way for targeted delivery of cytotoxic agents to the cancer vasculature.[284−286]

Tumor-induced angiogenesis is a consequence of ligation by extracellular matrix proteins to the $\alpha_V\beta_3$ integrin, which is highly expressed on many tumor cells. The $\alpha_V\beta_3$ integrin is one of the most specific of these exclusive markers, which is found on the luminal surface of the endothelial cells only during angiogenesis.[287,288] Targeting agents that are restricted to the vascular space during angiogenesis can recognize this marker.[288] A common binding motif in these matrix proteins is the amino acid sequence arginine−glycine−aspartic acid (RGD). Blocking tumor-induced angiogenesis by inhibition

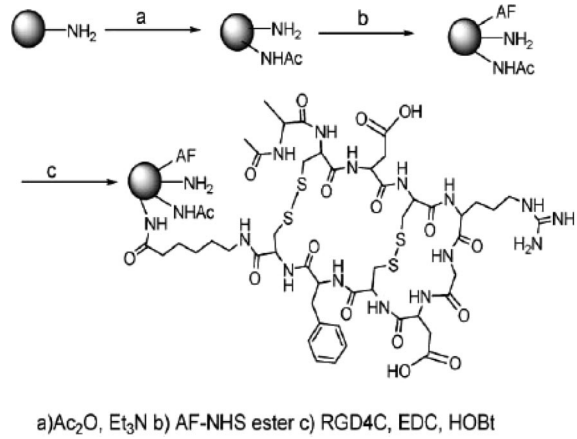

Figure 16. Schematic showing synthesis of RGD coupled 5.0G PAMAM construct. Adapted with permission from ref.[292] Copyright 2005 International Society for Chem Communication.

of the $\alpha_V\beta_3$ integrin is now a major target for cancer chemotherapy and many RGD-containing peptides and RGD peptidomimetics have been evaluated as antagonists of integrins.[286] Reports suggests that there has been an increasing interest in the synthesis of polymer—RGD conjugates for gene delivery,[289] tumor targeting,[290] and imaging applications.[291]

Dendrimers can be coupled to multiple $\alpha_V\beta_3$ selective ligands (RGD-4C) to target tumor-associated capillary beds and allow the delivery of cytotoxic agents to kill the new vessels. In the sole report of this type, Shukla et al. have described the synthesis and coupling of $\alpha_V\beta_3$ specific RGD to a fluorescence labeled 5.0G PAMAM dendrimer.[292] (Figure 16) The NH_2 terminated dendrimers nonspecifically bind with the cells because of the positive charge on their surface.[293] To improve targeting efficiency and reduce the nonspecific interactions, the amine terminated 5.0G PAMAM dendrimer surface was partially modified with acetic anhydride. They studied the binding properties and cellular uptake of these conjugates in HUVEC cells expressing $\alpha_V\beta_3$ receptors and found that addition of excess free peptide inhibited the uptake of the conjugate by cells, indicating receptor mediated uptake of the conjugate. Such dendrimeric conjugates have great potential as imaging agents and

chemotherapeutics agents as well as in combination with angiogenic tumor vasculature.[292]

6.4. Antibody/Ligand Guided Dendrimers

The cancer cell targeting approach described by Choi and co-workers is reminiscent of the immunoconjugate strategy in which distinct but linked entities are employed to first recognize, bind, and then subsequently modify a cancer compartment.[219] In addition to overexpression of other receptors, [287,288,177] tumor cells also express specific surface antigens. This exposes the biochemical language of cells, and thus helps in deciphering the route of antigen−antibody reaction to mediate targeting. Antibodies are excellent targeting tools because of their intrinsic ability to undergo feedback with a specific target. Antibodies can work against a tumor in a number of ways: they can either combine with specific antigens on the surfaces of malignant cells and make them susceptible to destruction by immune cells of the host, or they can direct them to self-destruct. An antibody can also attack the blood vessels or stroma that supports the tumor, and can also often be employed to block the action of growth factors that are needed for tumor growth. Likewise there is an array of ways by which antibodies can be made,[294] but all such approaches demand an exceptionally large amount of antibody, and getting the required amount presents many technical problems.[295]

The discovery of hybridoma technology has heralded a widespread exploitation of antibodies as targeting moieties. These can be conjugated directly to the drugs (immunotherapeutics), but direct coupling of drugs to this targeting anchor restricts the coupling capacity to a few drug molecules. Also, direct modification of an antibody molecule can impair its solubility and its biological activity, and thus hamper the targeting potential.[36] Following these difficulties, a subsequent alternative to the use of antibodies involved other naturally targeted carriers loaded with anticancer drugs, for example, immuno-liposomes, immuno-micelles, immunoconjugates, etc.[296] In addition, this strategy of the coupling of a drug device to a tumor specific anchor allows importing many drug molecules by means of the fewest ligands. A number of researchers have employed the latter concept with dendrimeric scaffolds to mount the final attack on cancer.[14,297]

Initially, Roberts et al. applied dendrimers as linkers in the coupling of synthetic porphyrins to antibody molecules. The resultant PAMAM−immunoconjugate retained approximately 90% of the activity of unmodified antibody. This group also reported that the entire conjugate was bound to the heavy chain of the antibody. This study along with a few others laid the foundation for establishing the potential of dendrimer immunoconjugates in tumor therapy and diagnosis.[298]

It is well-known that for radioimmunotherapy, the labeling of monoclonal antibodies with high specific activity is of prime importance, especially when radionucleotides with shorter half-lives are used. Keeping this in mind Kobayashi et al.[299] designed a conjugate containing the 4.0G PAMAM, which has 64 amines, conjugated with 43 molecules of TU−DTPA and intended to bind large numbers of radiometals to single antibody molecules. The resulting product was then conjugated with OST_7 (a murine-monoclonal immunoglobulin-G antibody). Later these immunoconjugates (^{111}In− or Gd−OST_7−PAMAM−TU−DTPA) were evaluated for specific activity, immunoreactivity, biodistribution, and tumor targeting ability in mice. Immunoreactivity of radiolabeled OST_7−PAMAM−TU−DTPA seems more (about 91%) than that of OST_7−TU−DTPA (about 84%), when compared with ^{125}I-labeled OST_7. Biodistribution studies showed that ^{111}In or Gd−OST_7−PAMAM−TU−DTPA cleared more rapidly from the blood and accumulated more in the liver than ^{111}In− or Gd−OST_7−TU−DTPA. In addition to better biodistribution profiles, PAMAM conjugates often showed more rapid clearance from blood.

The modification of neo-glycoconjugates with several covalently attached carbohydrate residues has also been reported to improve the immunogenicity.[273] T-antigen has been reported to be a cancer related epitope, and has been used as an antigen for the detection and immunotherapy of carcinomas.[300] The T-antigen−glycodendrimer binding properties were evaluated by ELISA and reported to reveal clear evidence of the binding between glycodendrimers and the mouse monoclonal antibody. Similarly, mAb-dendrimer conjugates have also been successfully employed to enhance the sensitivity of radioimmunotherapy, imaging, and immunoassays.[301−303]

Thomas et al. have reported the synthesis and in vitro biological properties of dendrimer-antibody conjugates employing two unusual antibodies, 60bca and J591, which bind to CD14 and prostate-specific membrane antigen (PSMA), respectively, and have employed these conjugates as model targeting molecules. The PAMAM dendrimer platform was also conjugated to FITC as a probe to investigate cell binding and internalization. It was found that the conjugates bound specifically to the antigen-expressing cells in a time and dose dependent manner and with affinity similar to that of the free antibody. The cellular internalization of dendrimer conjugate was confirmed by confocal microscopic analysis.[304]

To establish an effective nonviral gene delivery and a corresponding imaging method for tumors, Sato et al. synthesized oligonucleotide-carrier complexes. The 3′-biotinylated forms of the oligonucleotide (oligo-Bt) were ^{111}In-labeled through a diethylenetriamine pentaacetic acid chelate. ^{111}In-oligo was then mixed with positively charged 4.0G PAMAM or biotinylated 4.0G PAMAM dendrimers (PAMAM-Bt) to form electrostatic complexes. ^{111}In-oligo/PAMAM-Bt and ^{111}In-oligo-Bt were conjugated to avidin (Av) to obtain ^{111}In-oligo/PAMAM-Av and ^{111}In-oligo-Av, respectively. Then ^{111}In-oligo/PAMAM, ^{111}In-oligo/PAMAM-Av, ^{111}In-oligo-Av, and carrier-free ^{111}In-oligo were examined for internalization in vitro in human ovarian cancer cells (SHIN3). The biodistribution of ^{111}In-oligo−carrier complexes and of ^{111}In-oligo was examined in normal or i.p. SHIN3 tumor-bearing mice 2−24 h after intraperitoneal injection. ^{111}In-oligo−carrier complexes bound to the tumor cells were found to internalize at a rate of 34−56% at 24 h. In vivo, PAMAM, PAMAM-Av, and Av significantly enhanced the tumor delivery of ^{111}In-oligo by about 9.1%, 14.5%, and 24.4% of the injected dose per g of tissue at 24 h, respectively, as compared to delivery without a carrier (0.8% ID/g). In conclusion PAMAM-Av conjugates can effectively deliver ^{111}In-oligo to disseminated tumors.[305]

Though targeted therapeutics using antibodies offer a striking advantage over conventional approaches because of their potential for cancer specific delivery, there are problems such as decreased immunoreactivity and poor solubility associated with such conjugates. Patri et al. synthesized J591 anti-PSMA (prostate specific membrane antigen) antibody−dendrimer conjugates containing fluorophores on the den-

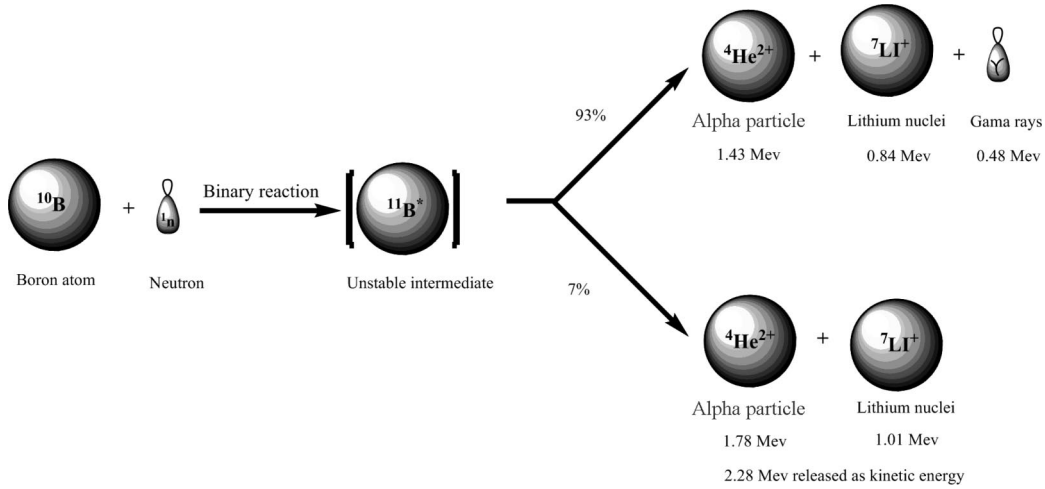

Figure 17. Schematic diagram showing basic principle involved in boron neutron capture therapy.

drimer and reported that by using a dendrimer as the carrier, these immunoreactivity and solubility issues can be resolved.[306] In addition, dendrimeric scaffolds have also been manipulated to achieve alternative release mechanisms.[307,308]

Most recently, Wu et al. have constructed a drug delivery device by covalently linking cetuximab (C225) via its Fc-region to a 5.0G PAMAM dendrimer containing the cytotoxic drug MTX. The resulting bioconjugate designated as C225−PAMAM−MTX contained 12.6 molecules of MTX per unit of dendrimer. The specific binding and cytotoxicity against the EGFR-expressing rat glioma cell line $F98_{EGFR}$ of this delivery device was successfully tested, and it was reported to have a high affinity for $F98_{EGFR}$ cells, with a 0.8 log unit reduction in its EC_{50} value. The biodistribution of C225−PAMAM−MTX was determined at 24 h after convection-enhanced delivery of ^{125}I-labeled bioconjugate in rats bearing implants of either $F98_{EGFR}$ or $F98_{WT}$ gliomas. At this time, 62.9 ± 14.7% ID/g of tumor was found to localize in rats bearing $F98_{EGFR}$ gliomas, and about 11.3 ± 3.6% ID/g of tumor was found to localize in animals bearing $F98_{WT}$ gliomas, which indicated specific molecular targeting of the tumor. But the therapy studies in $F98_{EGFR}$ glioma-bearing rats showed that animals that received C225−PAMAM−MTX, cetuximab, or free MTX had median survival times of 15, 17, and 19.5 days, respectively. These results were not significantly different from each other or from untreated control animals. This mixture of both positive and negative results indicated that specific targeting was but one of several requirements that must be met if an antibody-drug bioconjugate is to be made therapeutically useful.[309]

In addition to such applications, dendrimers have also been developed and introduced as a prospective platform for a multiprodrug. These structural dendrimers can release all of their tail units through a self-immolative chain fragmentation which is initiated by a single cleavage at the dendrimer's core. Incorporation of drug molecules as the tail units and an enzyme substrate as the trigger can generate a multiprodrug unit that will be activated with a single enzymatic-cleavage. Shamis et al. have synthesized the dendritic prodrugs with doxorubicin and camptothecin as tail units and a focal trigger which can be cleaved by catalytic antibody 38C2. The bioactivation of the dendritic prodrugs was confirmed in cell-growth inhibition assays employing the MOLT-3 leukemia cell line in the presence and the absence of antibody 38C2.[310]

Kobayashi and co-workers published data suggesting the use of a dendrimeric scaffold in the treatment of peritoneal carcinomatosis. Peritoneal carcinomatosis is a late stage in a variety of cancers, for which relatively no effective therapeutic modality exists. Gadolinium (^{157,155}Gd) is known to generate internal conversion electrons efficiently by neutron irradiation. Such electrons from neutron-activated Gd(III) are strongly cytotoxic, but only when Gd(III) atoms have been internalized into the cells. This group of researchers had developed a rapidly internalizing tumor-targeting arrangement to deliver large quantities of Gd(III) atoms into tumor cells. This arrangement was synthesized from 6.0G PAMAM dendrimer, biotin, avidin, and TU−DTPA. An in vitro internalization study revealed that Av-PAMAM−Gd showed accumulation internalization into human ovarian cancer, SHIN3 cells. This accumulation was 50 and 3.5 fold greater than Gd−DTPA and PAMAM−(TU−DTPA−Gd), respectively. In addition, MRI showed the accumulation of Gd(III) in the cells. The in vivo biodistribution study, performed in nude mice bearing intraperitoneally disseminated SHIN3 tumors, showed specific accumulation of Av-PAMAM−Gd in the tumor (103% ID/g), which was approximately 366 and 3.4 fold greater than Gd−DTPA (0.28% ID/g) and PAMAM−Gd (30% ID/g; Av-G6Gd), one day after intraperitoneal injection.[311] However, it is unlikely that Gd neutron capture therapy would be useful for the treatment of peritoneal carcinomatosis since this therapy would require irradiation of the entire abdomen. Also, reports over the past decade describe the role of dendro-immunoconjugates in boron neutron capture therapy in addition to drug therapy, gene therapy, and diagnosis in malignant situations. The following sections deal in particular with the role of dendrimers in boron neutron capture therapy as well as with antibody guided conjugates.

7. Dendrimers in Boron Neutron Capture Therapy (BNCT)

Boron neutron capture therapy (BNCT) is a binary approach to the treatment of cancer (Figure 17). For boron neutron capture therapy to be effective in curing cancer, a minimum ^{10}B concentration of 10−30 μg/g of tumor must be selectively delivered to the tumor.[37,312−315] To this end different ways of manipulating antibody conjugates of boron to achieve higher tumor localization have been studied.[316] The straightforward coupling of boron compounds to such

targeting anchors not only limits the yield of the desired conjugate, but also significantly impairs its solubility, biological activity, and targeting.[36] It is only after the passage of time and expenditure of much effort that the full potential of dendrimers is being realized in a wide array of fields. In the biomedical area, dendrimers have found promising applications for boron neutron-capture therapy.[317]

In 1994 the first boron-containing dendrimers were synthesized by Barth et al.[37] and Nemoto et al.[318] To fulfill the goal of using dendrimers for BNCT, Barth and Soloway investigated starburst, or as they are now called, PAMAM dendrimers, which were boronated by reacting them with an isocyanato-polyhedral borane. The monoclonal antibody IB16-6, which is directed against the murine B16 melanoma, was derivatized with *N*-succinimidyl 3-(2-pyridyldithio) propionate and reacted with the boronated dendrimers to yield stable immunoconjugates. In vivo biodistribution studies then revealed that these mAb−dendrimer conjugates had a propensity to localize in the spleen and liver. The zero-generation PAMAM dendrimers showed the lowest splenic and hepatic uptake, approximately 0.01% and 1.0%, respectively of the injected dose at 72 h. This pattern was found to relate directly to the molecular weight and terminal amine groups with higher generation dendrimers having five times more hepatic uptake than the zero-generation dendrimer. It was concluded that the random substitution of antibodies with large boron-rich moieties perturbs the mAb structure, resulting in increased competitive uptake of the conjugate by the liver and reduced tumor localization.[37] Bispecific mAb, which can simultaneously recognize both a tumor-associated antigen and a boronated macromolecule, was suggested as a possible alternative approach to eliminate this problem.[315,319]

Barth et al. chemically linked a heavily boronated starburst dendrimer (BSD) to EGF by heterobifunctional reagents. The rationale of their study was to determine the efficacy of boronated EGF, either alone or in combination with boronophenylalanine (BPA), as delivery agents for an EGFR positive glioma ($F98_{EGFR}$). For biodistribution studies rats were given an intratumoral injection of ^{125}I-labeled BSD−EGF. At 6 h, an equivalent amount of BSD−EGF was detected in $F98_{EGFR}$ and $F98_{WT}$ tumors. By 24 h 33.2% ID/g of EGF−BSD was retained by $F98_{EGFR}$ gliomas compared with 9.4% ID/g in $F98_{WT}$ gliomas, and the corresponding boron concentrations were 21.1 μg/g and 9.2 μg/g, respectively. Boron concentrations in normal brain, blood, liver, kidneys, and spleen all were at nondetectable levels (<0.5 μg/g). Two weeks after implantation of 10^3 $F98_{EGFR}$ or $F98_{WT}$ tumor cells, rats were given an intratumoral injection of BSD−EGF either alone or in combination with intravenous BPA and were irradiated 24 h after intratumoral injection. Untreated controls had a mean survival time (MST) of 27 ± 1 days, while irradiated controls had a MST of 31 ± 1 days. Animals bearing $F98_{EGFR}$ gliomas and receiving BSD−EGF boron neutron capture therapy had a MST of 45 ± 5 days compared with 33 ± 2 days for animals bearing $F98_{WT}$ tumors and receiving the same therapy. The animals that received BSD−EGF together with intravenous BPA had a MST of 57 ± 8 days compared with 39 ± 2 days for intravenous BPA alone. This study thus demonstrates a high molecular weight boron-containing delivery agent, BSD−EGF that could exclusively target receptor-positive tumor cells in vivo and produce an increase in survival time following BNCT.[320]

The gene encoding EGFR is overexpressed in human gliomas, and this by itself has been considered as a potential target for the specific delivery of therapeutic agents to brain tumors. Barth, Yang, and Wu have evaluated Cetuximab (IMC-C225), a monoclonal antibody directed against both the wild type and mutant isoforms of the epidermal growth factor receptors, as a boron-delivering agent for BNCT of brain tumors. This group had synthesized and studied mAb-dendrimer-boron conjugate (C225−5.0G-B) for BCNT. After 24 h of administration of boronated cetuximab conjugate (C225−5.0G-B), the mean boron concentrations in rats bearing these gliomas were found to be 92.3 ± 23.3 μg/g and 36.5 ± 18.8 μg/g, respectively. In contrast, the uptake of nontargeted boronated dendrimer (5.0G-B) was found to be 6.7 ± 3.6 μg/g, clearly briefing the sensitivity of Ab-mediated targeting. The mean survival time of rats receiving C225−5.0G-B was 45 ± 3 days compared to 25 ± 3 days for untreated controls. A further enhancement in MST to more than 59 day was reported on administering a combination of C225−5.0G-B with intravenous boronophenylalanine. These data were the first to demonstrate the efficacy of a boronated mAb for BNCT of an intracerebral glioma and hence can be regarded as a model construct for future studies using a combination of boronated mAbs and low molecular weight delivery agents.[41,321] Barth et al.[37] have also investigated the use of the same chimeric mAb cetuximab (IMC-C225) in the therapy of brain tumors. Their reports were analogous to that of Wu et al.[41]

Convection enhanced delivery (CED) is a powerful method to improve the targeting of macromolecules to the central nervous system by applying a pressure gradient to set up bulk flow through the brain interstitium during infusion. To evaluate CED systems as a means to improve the intracerebral and intratumoral uptake, Yang et al. studied a heavily boronated dendrimeric scaffold linked to EGF for neutron capture therapy in rats bearing a syngeneic EGFR glioma. The autoradiography and γ-scintillation counting data at 24 h after CED showed localization of 47.4% ID/g tissue in $F98_{EGFR}$ gliomas compared with 33.2% ID/g tissue after direct intratumoral injection. These observations strongly imply that the conjunction of the convection enhanced delivery and boronated dendrimeric scaffold approaches can be another effective way to deliver boronated EGF to EGFR(+) gliomas for boron neutron capture therapy.[322] Recently, the various factors that must be considered in bringing a variety of high molecular weight agents into clinical applications for BNCT were revieved by Wu et al.[323] Reports of Heldt et al.,[35] Qualmann et al.,[44] and Tansey et al.[247] have also described the use of functionalized dendrimers in covalently attaching boron atoms to dendrimers. Qualmann et al.[44] reported the synthesis of a lysine dendron with eight icosaedric dodeca-*o*-carboranes and 80 boron atoms. The terminals of this water-soluble dendrimer had free thiol groups that could be coupled to an antibody fragment to mediate the targeting.[44] (Figure 18) Shukla et al.[43] have reported on the construction of dendrimers with both a targeting ligand and PEG chains, which was composed of boronated dendrimers, folic acid, and PEG_{2000} chains. They observed lower hepatic uptake for the PEGylated conjugates. The same conjugate showed significantly enhanced tumor selectivity compared to the non-PEGylated antibody conjugates[37] as well as to the EGF conjugates,[40] which had been evaluated previously for their potential use as boron delivery agents for BNCT.

Targeting tumor vasculature in addition to the tumor cells themselves could be a useful way to enhance the efficacy of

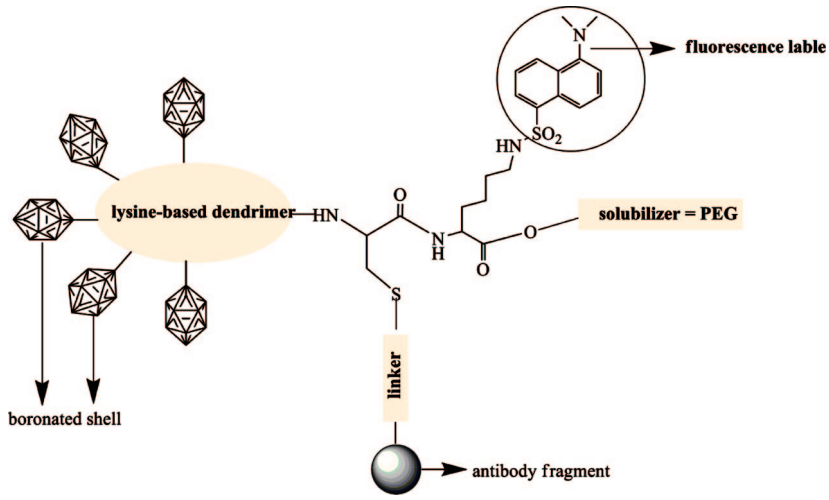

Figure 18. Example of PEG-antibody conjugated boronated lysine dendrimers. Adapted with permission from ref 44. Copyright 2005 International Society for Angewandte Chemie, International Edition.

BNCT. Tumor neovasculature is a likely but less-explored target for the boron neutron capture therapy of cancer. In a report Backer et al. have described the construction of a vascular endothelial growth factor (VEGF)-driven bioconjugate that potentially could be used to target up-regulated VEGF receptors, which are overexpressed on tumor neovasculatures. They synthesized a VEGF-boronated PAMAM dendrimer construct, and to facilitate in vivo analysis by fluorescent imaging, this construct was labeled with Cy5, a near IR dye. Using a mouse tumor model, they found that VEGF driven nanovehicles selectively accumulated in the marginal areas of growing tumors, where tumor neovascularization was the most active.[324] Dendrimeric systems synthesized by this method may enhance solubility and selective delivery of a payload to the target site. As a consequence of this, the necessary dose as well as the toxicity of the agent was substantially reduced (Figure 1f). These studies suggest that dendrimer technology, if cleverly manipulated, may prove to be a useful approach for cancer therapy.

8. Present Dendrimeric Vista and Future Prospects in Photodynamic Therapy (PDT)

Photodynamic therapy (PDT) has moved beyond the laboratory bench and into clinical practice. PDT involves the administration of a photosensitizer followed by activation with visible light of a specific wavelength. This generates highly reactive singlet oxygen species which are capable of producing lethal biological effects on tumor cells. This is associated with a series of photochemical reactions which finally result in an array of cytotoxic species. The nature, location, and amount of the cytotoxic species so generated along with the sensitivity of the target cells determine the outcome of PDT.

Since the initial approval of Photofrin as a photosensitizer for the treatment of bladder cancer,[325] the use of PDT for the treatment of cancer as well as non-neoplastic lesions has increased dramatically following advances in light applicators and photosensitizers. Photosensitizers are a vital component in PDT. At present, a numbers of photosensitizers have gained regulatory authorization, and some others are under clinical evaluation.[326] However, many of these photosensitizers have limited water solubility, cutaneous phototoxicity, and poor selectivity for tumors. It clearly follows from the above discussion on the properties of dendrimers and their use in overcoming solubility, nonselectivity, toxicity, and other problems affecting bioactivity that the dendrimeric scaffold can be effectively manipulated to improve PDT. Despite higher efficiency, the potential of unsubstituted aluminum phthalocyanine as a sensitizer for the PDT of cancer has not yet been fully exploited. This is a result primarily of its strong hydrophobic nature, which makes it difficult to formulate for in vivo administration. Brasseur et al. fabricated polymeric conjugates of aluminum pthalocyanine, which not only improved its water solubility but also its pharmacokinetics in EMT-6 tumor-bearing mice.[327]

5-Aminolevulinic acid, popularly referred to as Levulan represents one of the competent and approved class of photosensitizer. Protoporphyrin IX (PpIX) is produced in cells via the heme synthesis pathway from the substrate aminolevulinic acid (ALA) and can be used for tumor detection and monitoring or for photodynamic therapy. It is the only dendrimeric scaffold that can incorporate a high payload of bioactive molecules. Battah et al., using a convergent growth approach, synthesized a dendrimeric conjugate with 5-ALA acid residues covalently attached to the periphery by ester linkages and with amide bonds connecting the dendrons. This association selectively delivered the loaded photosensitizer upon hydrolysis selectively under physiologic conditions by generating PpIX. The potential of these 5-ALA acid ester dendrimer conjugates for PDT has been demonstrated against tumorigenic keratinocyte PAM 212 cell lines.[328]

Battah et al. have investigated the properties of three small molecular weight first generation dendritic carriers or "dendrons" in comparison to ALA. Cellular uptake followed by the release of ALA which was then converted to PpIX was observed for all dendritic derivatives. Efficient killing of cells was observed following illumination.[329]

Early reports demonstrated the applicability of PDT in treatment of skin malignancies,[330−337] particularly in conjunction with 5-ALA.[338−343] However, the hydrophilic nature of the 5-ALA molecule limits its penetration through the skin as well as cell membranes. Several approaches such as the development of more lipophilic molecules derived from 5-ALA and the incorporation of 5-ALA into lipophilic vehicles such as liposomes are currently under investigation to enhance 5-ALA penetration.[344] Chauhan et al. have

synthesized dendrimers with improved in vitro as well as in vivo transdermal fluxes.[51] These two concepts, if coordinated can produce a novel approach for employing dendrimers in PDT, in order to more effectively treat skin cancers.

Recent work of Kubat et al. demonstrates the pH-dependent behavior of porphyrins in the presence of PAMAM dendrimers. The binding of porphyrins to PAMAM dendrimers is controlled mainly by electrostatic interactions between porphyrins and the amine or carboxyl groups of PAMAM dendrimers. This process depends on the porphyrin peripheral functionalization and the pH value of the solution. These interactions between the PAMAM and porphyrins can stabilize aggregate structures. A positive charge of G5 induces the formation of H-dimers with the face-to-face arrangement of the anionic porphyrin units being preferred.[345]

Venosa et al. made an attempt to improve delivery of 5-ALA to tissue. They have investigated the use of dendritic derivatives capable of bearing several drug molecules and also evaluated the in vivo−in vitro efficacy of the first generation dendron, aminomethane tris-methyl 5-aminolaevulinic acid (containing three 5-ALA residues), in conditions of porphyrin synthesis. The porphyrin level induction by the dendrons in LM3 cells was found to be similar to that of equimolar concentrations of 5-ALA. Also the systemic and topical administration of the dendron to tumor-bearing mice induced higher levels of porphyrin than the widely investigated hexyl ester derivative. Despite the fact that the uptake of the dendron is similar to that of 5-ALA, it was also found that there was only limited intracellular release of 5-ALA residues. Hence, such reports require new accessibility studies and the use of methods such as those involving esterases to improve the design and application of these dendritic derivatives.[346]

Another recently published report suggested some ways to enhance the efficacy of PDT. One report on the photosensitizer 5-ALA suggested that the use of the iron chelator 1,2-dimethyl-3-hydroxy-4-pyridone in combination with 5-ALA further increased the yield of PpIX by delaying the conversion of PpIX to heme.[347]

In addition, Juzeniene et al. have reported that PDT is temperature-dependent and suggested that the photobleaching rate of PpIX in cells significantly increased with temperature during light exposure.[348] Photofrin-mediated PDT was shown to be a strong activator of vascular endothelial cell growth factor and COX-2 derived prostaglandins within the tumor microenvironment. Inhibitors that target these angiogenic and pro-survival molecules were proposed to further enhance the value of PDT.[349]

Larsen et al. have investigated the solvent mediated optimization of energy transfer properties in a series of Zn(II)−porphyrin dendrimers by means of exciton−exciton annihilation. Upon changing from a polar solvent (tetrahydrofuran) to a nonpolar solvent (3-methylpentane), the annihilation energy transfer rates increased by 28−44%. This is associated with a decrease of the hydrodynamic radius, which enhances the communication between the Zn(II)−porphyrin chromophores. As a consequence, the overall energy transfer efficiency was increased, thereby yielding complete annihilation between all the chromophores in the smallest generation dendrimer. This study showed how solvent control of the dendrimer size can be used to optimize the light absorbing and energy yielding capacity of the dendrimer.[350] Furthermore, such micellar formulations may significantly improve the circulation time as well as accumulation in hyperpermeable lesions, due to the EPR effect.[351,352] The report by Jang et al. describes the first model of dendritic phthalocyanine-incorporated polyion complex micelle formation for the enhancement of photodynamic efficacy. They prepared a polymeric micelle (DPcZn/m) system formed via an electrostatic interaction of anionic dendrimer phthalocyanine (DPcZn) and poly (ethylene glycol)-poly(L-lysine) block copolymers (PEG-b-PLL) for use as an effective photosensitizer for PDT. This system has an approximate size of 50 nm. Under light irradiation, either DPcZn or DPcZn/m exhibited an enhanced consumption of oxygen, generated reactive oxygen species, and produced an increase in photocytotoxicity dependent on the irradiation time. The photodynamic efficacy of the DPcZn was significantly improved by incorporation into polymeric micelles where after 60 min photoirradiation it characteristically exhibited more than 2-fold higher photocytotoxicity than the free DPcZn.[353]

PDT is now becoming more widely used for treating various types of tumors.[354−357] Moreover, the antitumor efficacy of PDT can be raised significantly by applying the notion of targeted immunoconjugates.[358−360] An alternative approach involves the entrapment of PEGylated tetraarylporphyrin in a vesicular carrier.[361] Such synthetic strategies were chiefly aimed at supplementing the properties of attached PEG chains as well as vesicular carriers in PDT. Such motifs seem however to be complicated, and the same goal can be achieved more easily by using more suitable dendrimeric scaffolds.

8.1. Dendrimer as Drug in PDT

Another approach has been to incorporate efficient photosensitizer porphyrins as a core within the dendrimer. This approach involved tumor localization of dendrimeric porphyrin conjugates using any of several receptor-mediated bioevents followed by irradiation of the dendrimeric porphyrin architecture. At the targeted site localized porphyrin core dendrimers convert light energy to chemical energy by generating singlet oxygen, which is an electronically excited species (Figure 19a). Studies show that $^1O_2$ can alter crucial biomolecules including DNA, proteins, and lipids, and is thus highly lethal (Figure 19b). Singlet oxygen in low concentrations can also act as a signaling molecule with several biological implications.[362]

Nishiyama et al. prepared the 3.0G-polyaryl ether dendrimer porphyrins with either 32 quaternary ammonium groups or 32 carboxylic groups at their periphery and evaluated this as a novel supramolecular class of photosensitizers for PDT. Depending on whether there were positive or negative charges on the periphery, dendrimer porphyrins showed different cell-association patterns, but both dendrimer porphyrins were eventually localized in membrane-limited organelle, which suggests endocytosis-mediated entry. This endocytosis-mediated cellular entry was more pronounced in the case of cationic dendrimers. The dendrimeric scaffold remained localized in lysosomes and other intracellular compartments. On the other hand PpIX, which is a hydrophobic and relatively low molecular weight photosensitizer, showed diffusion through the cytoplasm but not the nucleus. Both of the dendrimer porphyrins showed lower toxicity with regard to the disruption of membranes and intracellular organelles as compared to PpIX. Remarkably however, the dendrimers with peripheral quaternary ammonium groups achieved very much higher induced cytotoxicity against LLC

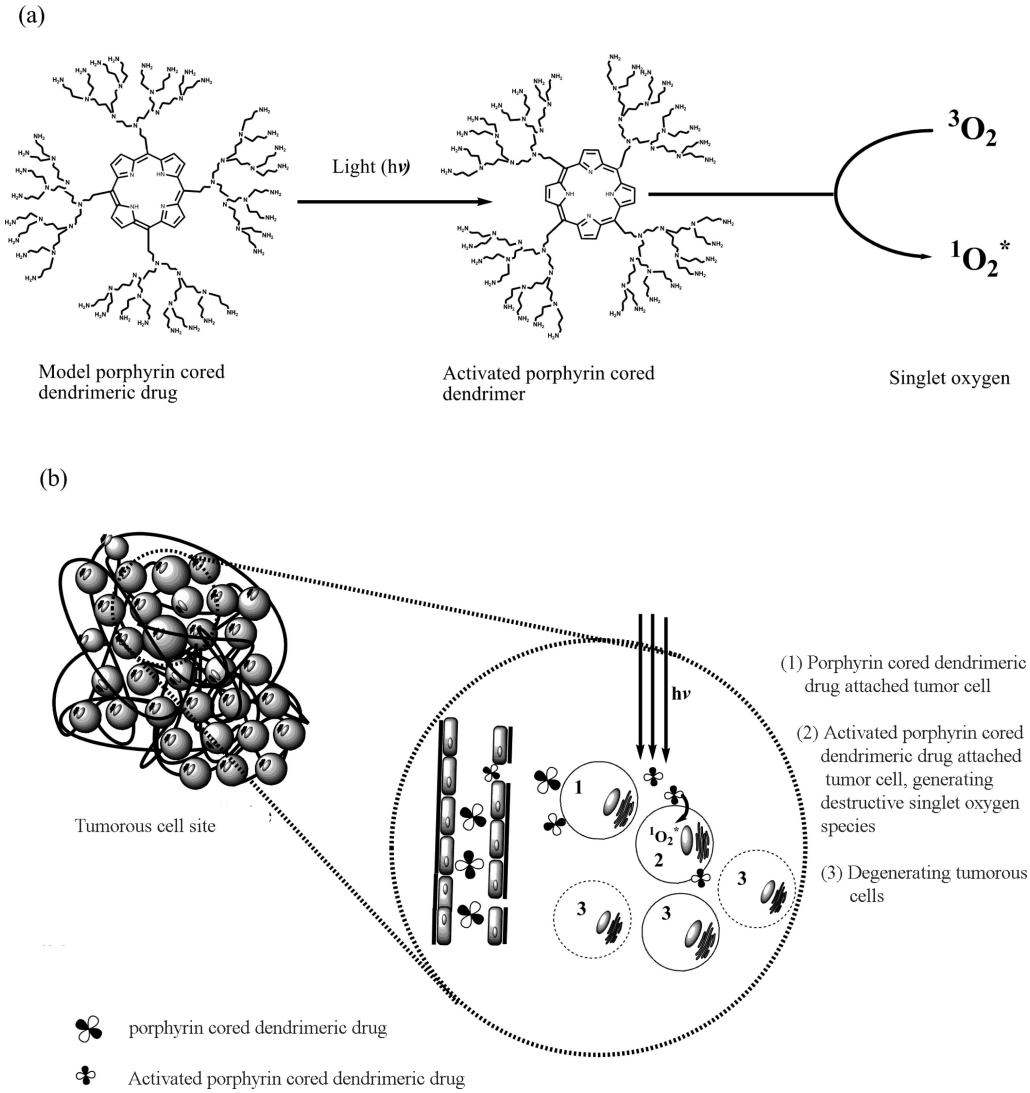

Figure 19. Schematic diagram showing druglike action of porphyrin-cored dendrimer: (a) generation of singlet oxygen by irradiation activated porphyrin-cored dendrimer; (b) bioevents involved in tumor wash-up by dendrimeric drug.

cells than did PpIX, which is in agreement with other reports.[73,115,116] Furthermore, both dendrimer porphyrins had about 140-fold lower dark toxicity as compared with PpIX, signifying their well-selective photosensitizing effect. A short dark toxicity is a prerequisite in photodynamic therapy since elevated dark toxicity causes undesired effects.[363]

To further explore the field, both a 3.0G aryl ether dendrimer porphyrin with 32 primary amine groups on the periphery and a pH-sensitive polyion complex micelle (PIC) composed of the porphyrin dendrimer and PEGylated poly-(aspartic acid), were evaluated as new photosensitizers for PDT in the Lewis lung carcinoma cell line. Electrostatic assembly resulted in a red shift of the Soret peak of the porphyrin core and enhanced fluorescence. As compared to the dendrimer porphyrin, a relatively low cellular uptake of the dendrimer porphyrin incorporated in the PIC micelle was observed, however the latter exhibited enhanced photodynamic effects. Moreover, the use of PIC micelles as a delivery system reduced the dark toxicity of the cationic dendrimer porphyrin, most probably due to the biocompatible PEG shell of the micelles.[364]

Dichtel et al. have reported that the porphyrin core dendrimer conjugates numerous two-photon absorbing chromophores. Upon irradiation at longer wavelength, this conjugate tends to proficiently generate singlet oxygen species.[365] Yamamoto et al. recently reported that metal-assembling dendritic phenylazomethines could also significantly enhance the electron-transfer reaction as a protein-like catalyst. The dendritic phenylazomethines having a cobalt porphyrin core could catalyze the CO_2 reduction at an applied potential over 1.1 V lower than what was needed for the catalysis. The reactions (reduction and oxidation) involving small molecules such as O_2, N_2, or CO_2 are of particular importance because they are fundamental resources for the production of various organic compounds. In addition, they also play a very important role in the energy circulation system of nature. This conversion generally involves very large activation energy values because of the multielectron transfer (m-ET) processes involved. Since dendritic phenylazomethines can construct their metal assembling structure around the core, they are expected to act as monostructured m-ET catalysts.[366]

Previous studies by the same groups revealed that the lanthanide metal ions assembling dendritic phenylazomethines with a cobalt porphyrin core act as an efficient catalyst for the CO_2 electrochemical reduction at a very low potential.[367] Cobalt tetraphenylporphyrin (CoTPP), which is a model of the core unit, is known to catalyze the reduction.[368,369]

It was suggested that the electron-exchange kinetics between the core and metal complexes must be very fast in order to accelerate the m-ET process. That the electron transfer time scale in the dendritic phenylazomethines complex is much faster than nanoseconds was confirmed by a fluorescence quenching experiment involving the zinc porphyrin core in dendritic phenylazomethines.[370]

Another approach for treating cancer involves two-photon excitation.[371] The applicability of single-photon (1-gamma) PDT is limited by the low specificity of the photosensitizer, which causes damage to healthy tissue near the diseased target. One solution of this issue is to use simultaneous two-photon (2-gamma) excitation with ultrafast pulses of near-IR light. Because of the nonlinear interaction mechanism, 2-gamma excitation with a focused beam is localized in three dimensions and thus allows treatment volumes on the order of femtoliters.[372]

9. Miscellaneous

9.1. Dendritic Architecture in Optical Fluroscence Imaging

Current progress with numerous biocompatible fluorescent molecules suggests that tumor biosensing could be perfected by using fluorescence detection techniques. These approaches would have additional advantages over other nonbiocompatible techniques such as radiation or chemical analysis. However, conventional approaches are limited by the absorption and scattering of light in the tissue.[373] Using an optical fiber placed into a preidentified tumor through a thin needle could provide a minimally invasive means to excite a fluorescent material and localize the emitted fluorescence and may avoid the problem of tissue absorption and scattering of light. Several biosensors based on one-photon fluorescence detection systems have been developed for the quantification of fluorescent materials in situ.[374,375]

In contrast to one-photon detection systems, two-photon systems can be used for the simultaneous detection of fluorochromes with a broad range of excitation wavelengths with a spatial resolution of only a few micrometers.[376] Some investigators have reported a two-photon optical fluorescence fibers (TPOFF) system which uses the same single-mode fiber to transport femtosecond laser pulses for excitation and for collection of the emitted tissue fluorescence.[377]

Thomas et al. have reported the applicability of the TPOFF probe for the real-time in vivo fluorescence measurement of a targeted fluorescent nanoparticle in live mice. They employed the 5.0G dendrimer (G5) conjugated to the targeting moiety (FA) and the fluorescent sensing agent 6-TAMRA (6T) as the carrier to target xenograft tumors in mice. A fiber optic TPOFF probe inserted deep into tissue using a thin gauge needle was sufficient to quantify the concentration of a fluorescent agent in the tissue. This technique has the advantage both of being minimally invasive and of being able to sense deep tissue in live animals. This can also provide quantitative knowledge of fluorescence targeted into a tumor, or could determine the *in situ* levels of a tumor-specific cellular molecule like oncogene protein product, or may help in monitoring tumoral delivery of a fluorescently tagged drug. It could also be used to identify apoptosis if used in combination with apoptosis-activated FRET reagents. The tumor fluorescence was documented in live mice at 30 min and 2 and 24 h with the help of a TPOFF probe. The authors found that G5-FA-6T showed selective accumulation in the tumor with maximum mean levels reaching 673 ± 67 nM at 2 h. On the other hand, the level of a control nontargeted conjugate (G5-6T) reached a level of only 136 ± 28 nM in tumors at 2 h and then decreased rapidly. This suggests that the TPOFF probe can be used as an effective detection system.[378]

The targeted (G5-FA-6T) and the control (G5-6T) dendrimer conjugates were synthesized using protocols similar to that reported previously.[379] The in vitro results were parallel and comparable to those reported earlier for conjugates in which fluorescein was used as the sensing agent.[214] Baker et al. have performed in vivo testing of dendrimer nanoparticles targeted by folic acid and having 6-TAMRA (6-carboxy tetramethyl rhodamine succinimidyl ester) as a fluorescent probe for labeling KB cell tumors. The fiber probe detected a 4-fold increase in tumor fluorescence in animals that received the targeted dendrimer. Their data demonstrate the utility of a technique that allows detection of fluorescence deep inside tumors using two-photon excitation through a fiber optic probe. Using the TPOFF probe, tumors containing as little as 0.3% fluorescent protein cells could be identified with sensitivity comparable to flow cytometry on isolated cells. These results also suggest that TPOFF can be used as a minimally invasive system for identifying tumor markers.[380] The monitoring might also be coupled to therapy if a photo-cleavable nanoparticle−therapeutic complex is targeted to cells and activated by the TPOFF to release the drug.[381] Recently, the Center for Ultrafast Optical Science, Michigan, developed a novel technique that may allow us to overcome the diffraction limit in conventional optical imaging to characterize multifunctional tectodendrimers.

9.2. Dendritic Nanocomposites in Cell Trafficing

Composites are a physical mixture of two or more organic as well as inorganic components which display improved properties as compared to the individual components. Molecular nanocomposites are an exciting new class of agents with immense medical applications. Their potential use in the radiation treatment of cancer is especially intriguing. These nanodevices were synthesized as monodisperse hybrid nanoparticles composed of radioactive guests immobilized by dendritic hosts. For instance, the delivery of β-radiation may be achieved by encapsulating radioactive gold into the nanocomposites. The solubility as well as compatibility of dendrimer nanocomposites is, to a great extent, dominated by the surface of the host molecules. Such architectures are spherical, monodisperse, and well defined, and options are in hand to synthesize them with varying surface properties.[382−384]

Recently the effect of the rare earth ions (Er^{3+}, Eu^{3+}, Gd^{3+}, Nd^{3+}, Tb^{3+}, Yb^{3+}) upon the fluorescent intensity of 3.0G PAMAM dendrimer with a peripheral 1,8-naphthalimide group has been investigated. The presence of the rare earth ions was found to evoke a photoinduced electron transfer leading to an enhancement in the fluorescence.[385] Kukowska-Latallo et al.[216] have reported the targeting of dendrimer nanoparticles to tumors in mice. Recently they extended their research by evaluating the biodistribution data of gold-dendrimer nanocomposites in B16 melanoma bearing mice. Their report extends the use of these nanodevices to exploit differences between normal and tumor microvasculature for mediating delivery specifically to the tumor site. The interaction of this machinery with the biological systems engages the size and charge characteristics of particles as well as their surface recognition potential. They have

synthesized nanodevices with different surface charges. Among all those tested, the positively charged ones were found to be toxic, resulting in the death of the mice.[386] This was believed to be the result of the ability of amine-functionalized dendrimers to form holes in as well as disrupt lipid bilayers.[110] The surface charge of nanoparticles was shown to affect its biodistribution in the B16 mouse melanoma model. In the case of neutral (acetylated) nanocomposites, rapid clearance was observed after one day except for the liver and spleen. Both the neutral and positively charged nanoparticles had low affinity for the brain and heart. The positive surface nanocomposites showed slightly higher accumulation at the tumorous site. They also observed that there was a relatively insignificant difference in the accumulation of positive and neutral surface nanoparticles in the blood, brain, heart, kidney, lungs, and pancreas. But, they observed a very significant difference in the affinity of these nanoparticles toward liver and spleen. Neutral surface nanoparticles showed high affinity for liver while the affinity of positive surface nanoparticles was more toward spleen.[386]

Optical imaging systems use fluorescence or bioluminescence for imaging the cells. Such structural imaging systems have been fused with molecular imaging technologies, and such mergers include PET, CT, SPECT, MRI, and magnetic resonance spectroscopy using molecular imaging probes.[387]

Previous reports of Arbab et al. brief the biophysical and metabolic properties of superparamagnetic iron oxide (SPIO) poly-L-lysine complex for magnetic cell labeling, viability, function, metabolism, and iron utilization, employing human cervical carcinoma cells.[388] A considerable number of reports are available on magnetodendrimers as a versatile class of magnetic tags.[389] Recently, Hider and co-workers have reported a novel hexadentate 3-hydroxypyridin-4-one-based dendrimer having iron chelating properties.[390] Bulte and co-workers have utilized a contrast agent, called a magnetodendrimer, which consists of iron oxide particles suspended within a dendrimer matrix that is efficiently taken up into cells and is optimized for magnetic properties favorable for imaging.[391] Recently, Tekade et al. have further broadened the avenues of such application by exploring dendrimers for dual bioactive delivery, wherin two bioactives of different properties are delivered simultaneously by employing solo dendrimer formulation.[392,393]

10. Conclusions and Future Medical Prognosis

Drug delivery is a critical aspect in formulation because proper selection of the delivery system can control the bioavailability, the concentration profile, and undesirable side effects. Dendrimers pose an exciting opportunity for chemists to fabricate macromolecular structures with a specifically tailored function. They are the same size as serum protein and hence are capable of directly entering into the tumor microvasculature. Dendrimers can be efficiently used to achieve pH-dependent release with a slower release of their payload under normal physiological conditions and a burst release at the acidic tumor site. In addition, the irritating behavior of polycationic dendrimers toward biomembranes creates transient nanoholes, which further helps in the exchange of payload across the biomembrane.

However, in reality, drug-loaded dendrimeric formulations release a considerable portion of the loaded bioactives at extra-tumorous sites after administration. To avoid such peripheral drug release, drug conjugation was explored. The abundance of free amine groups on the dendrimers' surface allowed easy covalent conjugation of drugs. But the open conformation of dendrimers still continued to exhibit higher hemolytic toxicity and low biocompatibility. PEGylation of the dendrimers produced biocompatible and long circulating nanocarriers with a sustained release effect. The liposomal "locked in" dendrimers were also reported to reduce dendrimer related toxicity as well as to make the system long circulating.

Through the sequential journey "simple dendrimer→drug conjugated dendrimers→ PEGylated dendrimers→liposomal 'locked in' dendrimers", dendrimeric development finally reached the concept of complete targeting called "hybrid dendrimers". At present, the ongoing research on curing cancer supports only the targeted attack concept. The flexibility of dendrimers toward terminal modification along with their high payload efficiency makes them an excellent carrier for targeted delivery. The anchoring of folic acid to dendrimers requires only simple conjugation chemistry, and there is much data supporting the success of folate-anchored dendrimers in both targeting anticancer bioactives and imaging modalities. A direct surface modification of dendrimers with hydrophobic moieties for either therapeutic or imaging purposes hinders its solubility. DNA-assembled folate conjugates and PEGylated hybrid dendrimers assist appreciably in overcoming such limitations. Glycodendrimers and RGD coupled dendrimers are an additional set of engineered nanocarriers with enormous targeting ability. Dendro-immunoconjugates decipher antigen−antibody reactions to mediate site-specific delivery and avoid the direct conjugation of bioactive agents to the antibody, thus avoiding solubility, bioactivity, and economic problems.

Dendrimers are also considered to be an effective tool for neutron capture therapy. Further, the usefulness of dendrimers in curing cancer has been increased with the advent of dendrimers bearing a druglike cancer killing property. The dendrimers synthesized based on this approach are a new class of PDT with reduced toxicity, and combining PDT with the concept of targeting has further enhanced its usefulness.

A detailed experimental study correlating the in vitro/in vivo behaviors of dendrimeric formulations can yield substantial information for developing a successful delivery device. The use of targeting requires the exploration of suitable spacers for conjugating the targeting moiety to dendrimers to affect stable ligand binding. Liposomal "locked in" as well as "tectodendrimers" still warrant further investigation to complete the available data. It is further anticipated that novel dendrimeric structures will be designed to act as effective carriers with a superior degree of sophistication, selectivity, and specificity toward cancer so as to bring cancer well within control.

Besides the pharmaceutical and medical fields, dendrimers have also made known their presence in many other fields. However, more convincing as well as exhaustive data on the safety and toxicity of dendrimers as well as their biofate are warranted in order to establish this nanocarrier as a more acceptable and pragmatic alternative, particularly in the field of oncology.

11. Abbreviations

10-HCPT	10-hydroxy camptothecin
TU-DTPA	2-(4-isothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid
Oligo-Bt	3′-biotinylated forms of the oligonucleotide

5-ALA	5-aminolevulinic acid
5-FU	5-flurouracil
6-CTMR	6-carboxytetramethylrhodamine
ADR	adriamycin
ODN	antisense oligonucleotide
Ara-C	arabinofuranosilcytosyne
Av	avidin
BNCT	boron neutron capture therapy
BSD	boronated starburst dendrimer
CPT	camptothecin
CoTPP	cobalt tetraphenylporphyrin
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
FITC	fluorescein isothiocynate
Gd	gadolinium
GA	glutaric acid
HePC	hexadecylphosphocholine
hFR	high affinity folate receptors
MRI	magnetic resonance imaging
MTX	methotrexate
MPEG	methoxy PEG
MP	methylprednisolone
mAb	monoclonal antibody
OST_7	murine monoclonal Immunoglobulin G antibody
GlcNAc	*N*-acetyl-glucosamine [Note: occurs only as GlcNAc8 in text]
NKR-P1	natural killer receptor-protein 1
OEGMA	oligo ethylene glycol methacrylate
PCR	percentage contrast enhancement
%ID	percentage injected dose
PBS	phosphate buffered saline
PDT	photodynamic therapy
PpIX	photosensitizer protoporphyrin IX
PPI	poly(propylene amine)
PGA	poly-(L-glutamic acid)
PAMAM	poly(amido amine)
PEG	poly(ethylene glycol)
PEO	poly(ethylene oxide)
PEI	polyethylenimine
PGDs	polyglycerol dendrimers
PGLSA	polyglycerol succinic acid
PLL	poly-L-lysine
PSS	polystyrenesulfonate
PIC	polyion complex micelles
PSMA	prostate specific membrane antigen
ST-PHPMA	semitelechelic poly[*N*-(2-hydroxypropyl)-methacrylamide]

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13. References

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