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REVIEW

Dendrimers in Gene Transfection

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Abstract—Dendrimers are a new class of nanocomposite materials. They are branching polymers whose structure is formed by monomeric subunit branches diverging to all sides from a central nucleus. The type of nucleus, attached monomers, and functional groups can be chosen during synthesis, which produces dendrimers of definite size, shape, density, polarity, branch mobility, and solubility. This review deals with problems of dendrimer molecular structures and capability of *in vitro*, *in vivo*, *ex vivo*, and *in situ* transfection of genetic material. Advantages and shortcomings of different types of dendrimers in this respect are discussed.

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Chemistry and technology of polymers traditionally focused on linear polymers. However, at the end of 1980s a new type of polymeric materials called dendrimers (from Greek "dendron" – tree and "meros" – branch) was obtained in groups of Tomalia, Newkome, and Vogtle [1-5]. Dendrimers are globular in shape with topological structure formed by monomeric subunit branches diverging to all sides from the central nucleus (Fig. 1) [6]. Properties of synthesized macromolecules can be precisely assigned in advance by choosing appropriate monomers and functional groups [2-8]. The following features can be distinguished in dendrimers: (i) multivalent surface containing numerous potentially active sites, (ii) envelopes surrounding the nucleus, and (iii) the nucleus with attached dendrons.

Starting in 1979, two main strategies have been used for dendrimer synthesis: divergent method in which dendron growth begins from the nucleus [2-10], and a convergent method in which already finished dendrimer branches join the nucleus [5]. According to the divergent method proposed by Tomalia's and Vogtle's groups [2-4], dendrimer synthesis includes association of monomeric modules in a radial structure, from one branch to another, following definite rules. In particular, the dendrimer increases in size from one layer to another (from one generation to another). According to the convergent method proposed by Hawker and Frechet [5], branches that at the final stage of the process join the nucleus and form the dendrimer are grown first in the course of dendrimer synthesis.

The divergent method is successfully used for production of large amounts of dendrimers, because each step, adding one dendrimer layer (generation) results in doubling the dendrimer mass. Divergent synthesis is indispensable for synthesis of many dendrimer generations. However, incomplete growth and some side effects result in appearance of imperfect samples. It is very difficult to purify dendrimers obtained by this method because they are structurally similar to the side products.

Advantages of the convergent method include, first of all, easiness of purification of intermediate products and, second, considerable opportunities for design of branches of different groups. Rapid and inexpensive synthesis of a few dendrimer generations is possible using the convergent method. A significant disadvantage of this method is the great difficulty of branch consolidation around the focal point during synthesis of many dendrimer generations. Special attention in development of the convergent method is now given to methodology for synthesis of bi- and hypernuclear dendrimers and branched monomers. These technologies suggest combination of divergent and convergent methods of synthesis [2-12].

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An interesting property of dendrimers is the dependence of their solution viscosity on molecular mass, which is different from that for linear polymers [13]. Solution viscosity for linear polymers is described by the exponential function $\eta \sim C^N$, where C is polymer concentration and N is the exponential parameter ($1 < N < 10$), but viscosity of dendrimer solutions is described by the linear function $\eta = \eta_0(1 + k\varphi)$, where k is constant and φ is the disperse phase volume fraction. Thus, on increase in dendrimer generation (molecular mass), at a certain point the viscosity in solution begins to decrease. This effect is the result of globular dimensions of high dendrimer generations [14].

Now with regard to modifications, over 100 kinds of dendrimers have been synthesized [2-16]. The five most widespread families can be distinguished among them.

Polyamide amine (PAMAM) dendrimers [7] are based on the ethylenediamine nucleus, and their branches are designed of methyl acrylate and ethylenediamine. Half generations of PAMAM dendrimers have surface carboxyls, while complete generations have surface amino groups. At the present time there is a great choice of PAMAM dendrimers with quite different types of surface groups. *Polypropyleneimine (PPI) dendrimers* [3, 8, 13] are based on the butylenediamine nucleus and polypropyleneimine monomers. Besides PPI, another popular abbreviation of these dendrimers is DAB (diaminobutyl), based on the name of the nucleus. They are now commercially available. *Phosphorus dendrimers* are synthesized by the group of Majoral and Caminade [15]. Phosphorus atoms are present in the nucleus and branches of these dendrimers. *Carbosilane dendrimers* are based on a silicon nucleus and have ammonium or amino groups at the periphery [16]. *Polylysine dendrimers* are based on the amino acid lysine and have polylysine branches and surface groups [17]. They are now commercially available.

Owing to their peculiarities, dendrimers have become useful in gene therapy, one of most promising trends in therapy of numerous diseases. Adenovirus gene therapy is officially permitted in China and thus it is already included in practice of clinical medicine despite its side effects and potential risks.

Specific and efficient delivery of genetic material to ill organs and tissues as well to certain cell populations can be achieved using numerous viral and non-viral delivery systems (often named vectors), each of which has its own advantages and disadvantages. Viral vectors are most efficient but their shortcomings are high immunogenicity and carcinogenicity *in vivo* [18, 19]. Compared to viral systems, synthetic (non-viral) ones are characterized by lower efficiency but higher flexibility and safety [20].

Gene therapy is based on two conceptually different approaches. The first suggests delivery of plasmid DNA or corresponding constructs for expression of the gene of interest under control of an appropriate promoter, which will result in increase in the target activity, i.e. in production of a protein playing the role of a drug. The other provides for delivery of oligomeric genetic material like antisense oligodeoxynucleotides or siRNA (short interfering RNA) causing a decrease in the target activity, which finally results in inhibition of harmful mRNA expression and/or synthesis of harmful proteins. Such key factors are considered in choosing an approach as the number of genes involved in pathogenesis (monogenetic/polygenetic), therapy duration (temporal or permanent), efficiency of approach, suitable targets, and methods for regulation of genetic drugs. None of the presently available viral vectors meets all these requirements, and therefore the search of non-viral synthetic vectors is an urgent problem [20-22].

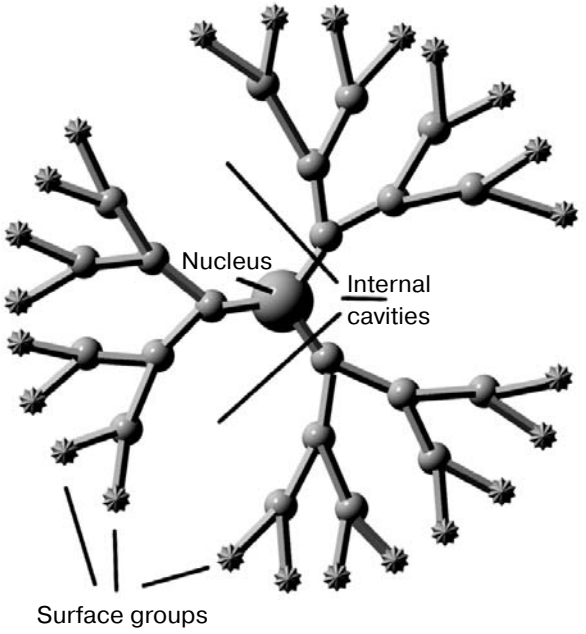

Fig. 1. Dendrimer structure.

To understand the necessity of nucleic acid delivery systems, we shall briefly describe barriers for nucleic acid (NA) delivery within organisms. In most cases introduction of free nucleic acid is accompanied by its enzymic degradation in the organism [23], which makes necessary the existence of systems for plasmid NA packing and transport [22]. Viral and synthetic vectors are designed for this. Moreover, vectors help in NA delivery to zones necessary for its localization and provide for efficient intracellular transport, usually to the nucleus [20]. Most often, the packing of NA is provided by electrostatic interaction of its anionic phosphate groups with positive charges of the synthetic vector, which results in complex formation.

Now NA complexes with liposomes and different cationic linear polymers are the most widely used non-viral vectors. Complexes with liposomes are called lipoplexes, and those with linear polymers are called

polyplexes. Dendrimers with positively charged groups are also able to bind NA, and by analogy with lipoplexes and polyplexes they are called dendriplexes [20]. This class of polymers has structural advantages for gene transport. Dendrimers are monodisperse, stable, and are characterized by relatively low viscosity at high molecular mass and numerous end groups that can be ionized, which means that they can efficiently bind a large amount of genetic material. It was found that the amount of DNA-bound surface groups doubles as the dendrimer generation increases [24]. This also influences the nature of complexes formed by different generations. According to a proposed model [25], there are sites of two types: binding (linker) and tightly packed. Along with increase in the dendrimer generation, the increase in the number of tightly packed sites, in which DNA is wrapped around the dendrimer, is observed (Fig. 2).

In parallel with investigations of dendriplex nature, experiments were begun on delivery of genetic material into cells and tissues using dendrimers. Haensler and

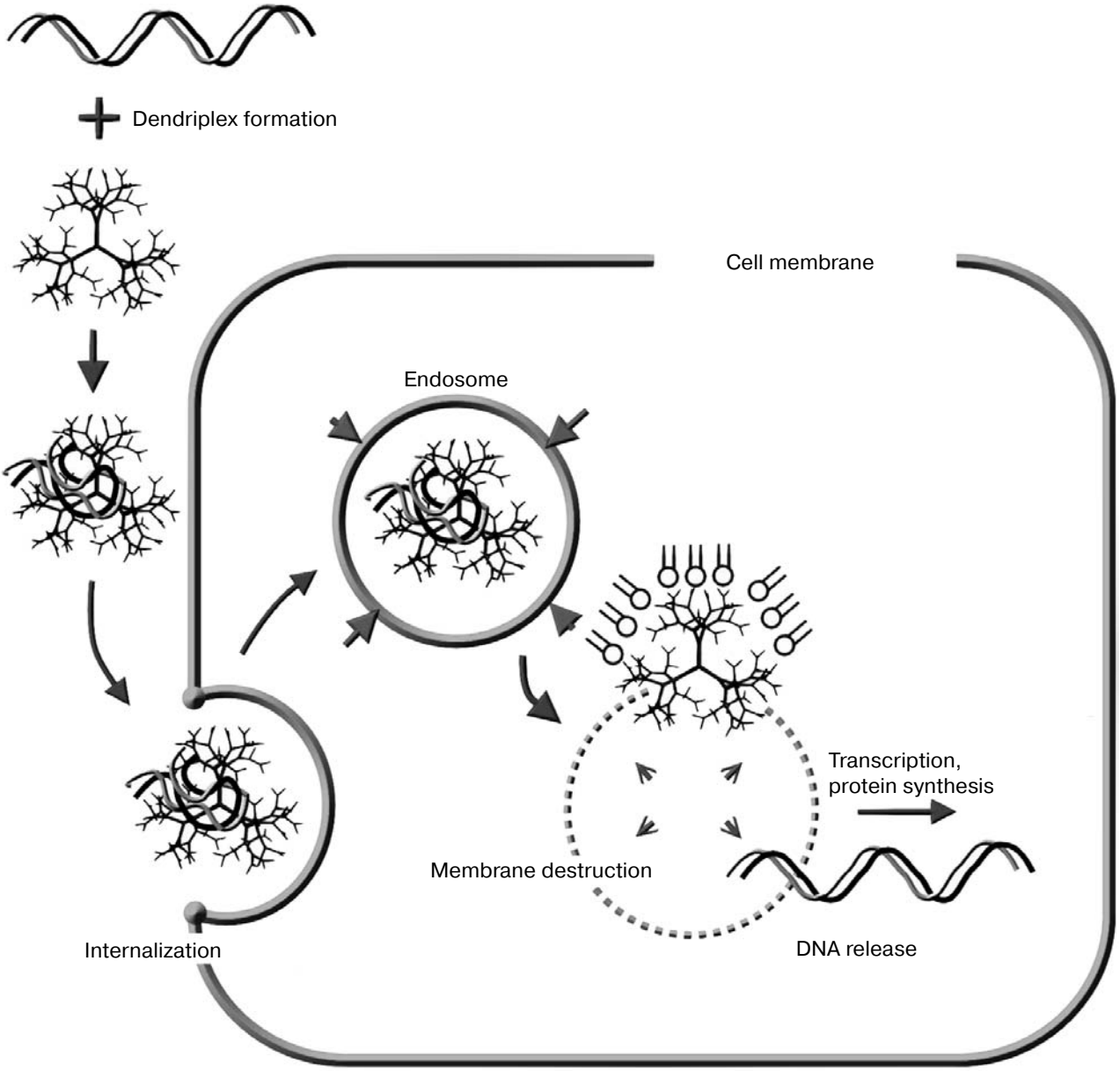

Fig. 2. Genetic material transfer using dendrimers [26]. First, the dendrimer forms *in vitro* a complex with nucleic acid. Then the complex is added to cells *in vitro* or is introduced into animals *in vivo* or *ex vivo*. The dendriplex can be delivered to their localization loci by the blood flow. After that the dendriplex is internalized by cells, and dendriplex-containing endosomes are formed. Deprotonation of dendrimer surface groups, dendrimer destruction, and nucleic acid release occurs when the pH changes from 7.4 (extracellular value) to 5.5 (intracellular value). Simultaneously, the endosomes undergo lysis and free nucleic acid is released into the cytoplasm.

Szoka [27] were the first who reported in 1993 that plasmid DNA containing luciferase and β-galactosidase genes can be delivered into cells using PAMAM dendrimers. In 1996, Baker et al. [28] published results of investigation of efficiency of genetic material transfection using dendrimers with different cell lines. It was shown that protonated dendrimers in a wide range interact with negatively charged plasmid DNA, and the formed complex is stable under physiological conditions even in the presence of SDS. Transfection efficiency was dependent on the dendrimer type in the presence of DEAE-dextran. In some situations, efficiency of transfection using dendrimers exceeded 10-100-fold the efficiency of commercial cationic lipids [28].

Later the same group studied *ex vivo* delivery of genetic material using dendrimers [29]. Whereas in *in vivo* experiments the gene–carrier complex is directly introduced into the organism, in *ex vivo* experiments cells are isolated from an organism, modified, and then are introduced back into the organism. Such approach was used for genetic material transport into myocytes. It was shown that DNA could be delivered into cardiomyocytes along with dendriplexes directly introduced into a donor's heart. Preliminary treatment with serotonin and prolonged time of dendriplex incubation enhanced delivery of genes and their expression in cardiac transplants. In this case, fifth generation PAMAM dendrimers were very efficient [29].

Dendrimers can be carriers of genes intended for treatment of malignant tumors. Gene therapy is one of the perspective approaches for therapy of cancer with account for limitations used for such generally accepted methods like chemo- and radiotherapy. Tumor growth during gene therapy can be stopped using angiogenesis control [30]. In studies of dendrimer efficiency as carriers of mammary gland cancer genes, PAMAM dendrimers were associated with 36-mer anionic oligomers for delivery of angiostatin and genes of tissue inhibitor of metal protein kinase (TIMP-2) [31]. The ability of the dendriplex to provide for gene transfer to tumor of mammary gland cancer was first checked *in vitro* using the plasmid codon for green fluorescent protein. Then the efficiency of angiostatin and *TIMP-2* gene transfer to the tumor localization sites was analyzed *in vitro*. It was found that gene transfer significantly decreased endothelial cell proliferation by blocking recovery of endothelial and cancer cells. In the final step of this work, gene transfer into the tumor region was checked on mice *in vivo*. The results were encouraging [31].

PAMAM dendrimers were used to deliver genetic material into cells of human skin [32]. This approach is rather promising for prolongation of NA effect on skin cells and enhancement of skin cell permeability for genetic material. Unlike other carriers, dendriplexes did not lose the ability to penetrate through human skin even after their application and following washing off. Simultaneously the possibility of *in vitro* dendriplex transfer from poly(D,L-lactid-co-glycolide) to cells through membranes was checked [33]. It was found that dendriplexes passed through membranes and appeared in the cells under study. In the presence of collagenase, retardation of dendriplex release *in vitro* was observed. The conclusion concerning the efficiency of genetic material transfer into skin cells *in vitro* with involvement of dendrimers was confirmed by *in vivo* studies. Application of dendriplexes containing a staining pigment gene on the skin of depigmented mice resulted in emergence of stained regions indicative of transfer of the gene [32, 33]. PAMAM dendrimers appeared to be efficient in *in vitro* delivery of the hypoxia-inducing *VEGF* gene into rat insulocytes [34].

Compared to PAMAM dendrimers, high generation PPI dendrimers were more toxic and less efficient for transfer of genetic material [35, 36]. However, Zinselmeyer et al. [37] have shown that generations of PPI dendrimers can be successfully used for genetic material transfer into cells. It was shown that DNA was completely condensed by high generations of PPI dendrimers (generations 3-5) and was only partially condensed by low generations (1-2). Interestingly, complete DNA condensation was not necessary for efficient gene transfer. Low generations not only more efficiently transported genetic material, but they were less toxic compared to dendrimers of high generations [37]. It was shown that DNA delivery by PPI dendrimers was made possible mainly for liver but not for lung tissue [38]. Kuo and Lin [39] compared the efficiency of genetic material delivery by PAMAM dendrimers of the 2nd and 5th generations and by PPI dendrimers of the 2nd and 3rd generations into human macrophages and mouse fibroblasts. PPI dendrimers were more efficient than PAMAM dendrimers in delivery of plasmid pSG5lacZ DNA encoding the *lacZ* gene for β-galactosidase.

Phosphorus dendrimers are another class of dendrimers for potential delivery of genetic material [40]. These dendrimers are stable in water over a broad pH range. After surface group modifications (replacement of anionic groups by positively charged ones), phosphorus dendrimers showed *in vitro* significant efficiency for luciferase gene transfection into 3T3 cells. Transfection efficiency increased linearly as the generation increased from 1st to 3rd and then reached a constant level. It is interesting that in the presence of 10% blood serum dendrimer cytotoxicity decreased, while transfer efficiency increased [40].

Currently, in addition to DNA delivery into cells for gene syntheses, delivery of single-stranded antisense oligodeoxynucleotides (ODN) began for mRNA blocking and thus for blocking translation of harmful disease-encoding genes [41]. Transcription can be also disturbed by triplex-forming oligonucleotide binding in the region of the DNA target gene promoter [42]. Efficient delivery

systems are necessary for ODN transport because ODN themselves very poorly pass through cell membranes and are quickly destroyed by cellular nucleases. Dendrimers were used for increase in efficiency of ODN delivery into cells. Bielinska et al. [43] showed that PAMAM dendrimers can be used as agents for antisense ODN transfection. They achieved inhibition of corresponding gene expression at picomolar ODN concentrations. Dendrimers in these concentrations showed no cytotoxicity. Hollins et al. [44] estimated potential of low generations (2nd and 3rd) of PPI dendrimers for cell delivery of antisense ODN for blocking epidermal growth factor expression in the epidermoid carcinoma cells. This receptor plays a central role in initiation and development of mouth, lung, and brain tumors [45]. Both inhibition of expression and inhibition of corresponding cell growth were observed in response to dendriplex introduction. Both dendrimer generations enhanced approximately 10-fold the efficiency of ODN delivery. It is not surprising that the second generation was less toxic than the third [44]. Santhakumaram et al. [46] studied efficiency of five generations of PPI dendrimers in delivery of the 31 bp long triplex-forming ODN directed against *c-myc* oncogene in mammary gland and prostate cancer cell lines. Oncogene *c-myc* is involved in cell proliferation. Results have shown that dendrimers were able to improve 14-fold the ODN delivery compared to free ODN. Dendrimers were not cytotoxic in the range of concentrations used. Efficiency of dendrimer generations depended on the transferred genetic material. It was shown that PAMAM dendrimer is able to form stable complexes with TAR region of HIV-1 mRNA and prevent Tat peptide interaction with this site [47]. The Tat interaction with TAR region of HIV-1 mRNA is necessary for production of viral transcripts and viral proliferation. Substances binding to the TAR region in mRNA and preventing its binding to Tat are gene-engineered drugs for inhibition of HIV-1 replication. PAMAM dendrimers improved delivery of ANTITAR ODN for binding to the TAR region. Carbosilane dendrimers were successfully used for binding different antisense ODN against HIV-1 [48-51]. It was shown that dendriplex formation between carbosilane dendrimer and ODN prevents their interaction with serum albumin [49-51]. As is known, the interaction of NA with blood serum albumins along with their degradation by blood proteases is the main barrier for free NA delivery to damaged organs and tissues during gene therapy. It was also shown that the use of dendriplexes based on carbosilane dendrimers resulted in lowering the antigen p24 (HIV-1 marker) level in the MT-2 cell line, which is indicative of efficiency of antisense ODN transfer using carbosilane dendrimers [51].

Investigation of mechanisms of gene activity regulation has recently revealed a new mechanism of gene expression inhibition – RNA interference, i.e. the ability of double-stranded RNA to stimulate specific degradation of mRNA target whose sequence is complementary to one of the double-stranded RNA strands. When long double-stranded RNA enters a cell, they undergo cleavage by endonucleases to short, 19-21 bp double-stranded fragments [52] with two protruding nucleotides at the 3′-ends of the strands. These short duplexes called siRNA form within a complex with proteins catalytic structures causing directed degradation of complementary target mRNA. RNA interference is now actively used for gene expression regulation and as a method for investigation of functional genomics of eukaryotes. As for other NA, one of the main limitations of the applicability of RNA interference for gene therapy is the problem of siRNA delivery into cells. Carbosilane dendrimers of the first and second generations were used for siRNA delivery into mononuclear cells of peripheral blood and into SupT1 lymphocyte cell line [48]. The dendriplexes showed low toxicity and high efficiency in siRNA delivery into cells. This resulted in significant decrease in HIV-1 replication. Posadas et al. [53] used siRNA delivery to postmitotic neurons with involvement of carbosilane dendrimers for investigation of the function of hypoxia-inducible factor α-1 in chemical hypoxia-mediated neurotoxicity and found that carbosilane dendrimers were efficient and nontoxic carriers for this purpose: the delivery resulted in pronounced blocking of factor α-1. Zhou et al. [54] checked the possibility of using PAMAM dendrimers for *in vitro* siRNA transfer. It was found that PAMAM dendrimers of 7th generation efficiently transferred siRNA for blocking transcription of the *GL3Luc* gene. Inoue et al. [55] suggested the use of polylysine dendrimers for siRNA transfer and found that dendriplexes based on polylysine dendrimers and siRNA exhibit low toxicity and cause efficient knock-down of the glyceraldehyde-3-phosphate dehydrogenase gene (EC 1.2.1.12) in several cell lines. In addition, efficient knock-down of the phosphoenolpyruvate carboxykinase (EC 4.1.1.32) gene was observed (this enzyme is rate-limiting for glyconeogenesis) as well as decreased glucose production in rat hepatoma H4IIEC3 cells.

Higashi et al. [56] studied NA binding by PAMAM dendrimers of the 3rd generation having oligo-L-lysine residues (from 5 to 40 polypeptide fragments) on their surface. Experiments showed the existence of strong interaction in the dendrimer–NA system, which was enhanced with elongation of the fragments. During development of these works, dendrimers completely consisting of lysine or ornithine residues were synthesized [57]. These polypeptides had no dendrimer-specific globular structure but were sufficiently branched and had positive charges on their surface, which made possible their efficient binding to genetic material. Simultaneously, their low cytotoxicity was detected. These results indicate [57] that such dendrimers are promising for genetic material transfer *in vivo*. Okuda et al. [58] studied the efficiency of genetic material transfer using dendrite polylysines.

Experiments on four cell lines and different dendrimer generations showed the dependence of transfer efficiency on dendrimer generation: the higher the generation, the better the effect. Vlasov et al. [17, 59] synthesized the 3rd generation of dendrimers based on polylysine and its derivatives. It appeared that the level of genetic material delivery by such dendrimers was low. To enhance this effect, dendrimers of higher generations as well as those with covalently attached antibiotic chloroquine and/or nonadecapeptide JTS-1 were synthesized. These agents stimulated dendrimer penetration into cells or were lysosome-destroying agents and thus enhanced the efficiency of genetic material transfer into cells. Eom et al. [60] synthesized α-, β-, and ε-dendrite polylysines and studied transfer by them of antisense ODN. *In vitro* experiments on HeLa cell lines revealed high efficiency and low cytotoxicity of these dendrimers. Li et al. [61] suggested the use of polylysine-polylactide dendrimers and studied transfer by them of plasmid DNA to hepatocarcinoma cell lines. It exceeded that for the standard polyethyleneimine carrier. To improve the genetic material transfer by dendrite polylysines, Coles et al. [62, 63] proposed polylysine dendrimer conjugation with TAT peptide, providing for dendrimer penetration into a cell, and with the nuclear localization signal peptide. Thus, these conjugates provided for the rapid penetration of dendriplex into cells and rapid delivery of genetic material to the nucleus.

The optimization of genetic transport using PAMAM dendrimers was investigated. Efficiency of transfection using PAMAM dendrimers was significantly enhanced by their thermal treatment, some of the branches being destroyed by solvolysis. This process was accompanied by increased strand mobility and retention of molecular dimensions. Such activated molecules were called fractionated or degraded PAMAM dendrimers, and production of commercial carrier Superfect™ on their basis began. Tang et al. [64] found that fractionated dendrimers showed higher efficiency in NA transfer compared to intact ones. However, there is a certain optimal level of degradation, above or below which a decrease in transfer efficiency is observed. Turunen et al. [65] checked the efficiency of liposomes and cationic polymers for gene transfer to smooth muscle and endothelial cells *in vitro*. The aim of this work was choosing the most appropriate carriers for gene delivery to the rabbit carotid artery *in vivo*. Fractionated PAMAM dendrimers of 6th generation exhibited the highest efficiency *in vitro* and *in vivo*. Ohashi et al. [66] used fractionated PAMAM dendrimers for gene delivery into chondrocytes. Gene delivery into chondrocytes is a new approach to treatment of chondroid tissue diseases and cartilage degradation. In the case of gene delivery into chondrocytes, *in situ* expression of a protein necessary for normal cartilage activity begins. Fractionated dendrimers appeared to be efficient carriers for gene delivery into chondrocytes. There were also attempts to use fractionated PAMAM dendrimers for gene delivery in the case of lung diseases. Unfortunately, dendrimers were inefficient for pulmonary gene delivery *in vivo* [67]. Praetorius et al. [68] compared *in vivo* transfer of plasmid DNA encoding green fluorescent protein into Corti's (spiral) organ of the mouse ear cochlear labyrinth by different carriers and showed the fractionated dendrimer efficiency for this purpose. On the whole, fractionated dendrimers significantly increased efficiency of delivery due to their higher mobility and compactness of formed dendriplexes.

Obviously, compactness of amino groups on the dendrimer surface is important for transfer efficiency [69]. The number of dendrimer surface groups, its diameter, and its conformation depend on the nucleus. Zhang et al. [70] synthesized PAMAM dendrimers with nuclei of three types: pentaerythritol, inositol, and trimesyl. All dendrimers had similar chemical composition but different architecture. In the case of dendrimers with pentaerythritol and inositol groups, the 5th generation was minimal for efficient DNA delivery, whereas in the case of the trimesyl nucleus it was the 6th generation. The NA transfection using PAMAM dendrimers based on the pentaerythritol nucleus was studied on several cell lines, and it was found that such dendrimers exhibited higher transfection efficiency and lower cytotoxicity compared to polypropyleneimine and unmodified PAMAM of 5th and 7th generations [71].

Surface modification of dendrimers can increase the efficiency of genetic material delivery and decrease cytotoxicity of complexes. Luo et al. [72] synthesized PAMAM dendrimers of the 5th generation with surface groups based on polyethylene glycol 3400. The aim of their work was the development of cheaper dendrimers than fractionated ones with preservation of their transporting features. The new dendrimer exhibited 20-fold improvement in gene delivery compared to fractionated ones. This effect can be explained by non-electrostatic (possibly by hydrogen bond) interactions between polyethylene glycol and nucleic acids, which resulted in easier NA release from such dendriplexes. Moreover, cytotoxicity of dendrimers based on polyethylene glycol was below that of standard analogs. Russ et al. [73] studied the possibility of construction of small pseudodendrimers based on the oligoethyleneimine nucleus (800 Da). Such pseudodendrimers exhibited high efficiency both *in vitro* and *in vivo*. Mice with inborn tumor received dendriplexes, and gene expression in different organs and tissues was studied. The results showed that dendriplexes were localized mainly in positions of malignant tumors. Huang et al. [74] synthesized PAMAM dendrimer conjugated with polyethylene glycol and the peptide transferritin. The peculiarity of this peptide is its ability to penetrate into the brain from the blood by successfully passing through the blood–brain barrier. Investigations using ^{125}I and fluorescence microscopy showed that this conjugate was able to penetrate successfully from blood into brain and transfer DNA for expres-

sion of exogenous luciferase gene in mouse brain *in vivo*. Genetic material transfer in this conjugate was tenfold higher than in generally accepted carriers and double compared to PAMAM/DNA and PAMAM-PEG/DNA complexes. Theoharis et al. [75] studied genetic material transport to activated vascular endothelium using the PAMAM dendrimer conjugates with antibodies to E- and P-selectins. As is known, adhesion molecules E- and P-selectins are expressed on the walls of activated endothelial cells. This provided for more efficient and localized DNA delivery to CHO-E cells and activated endothelial cells of the leg subcutaneous vein *ex vivo*.

To enhance NA binding to the dendrimer surface, modification of the latter was studied using hydrophobic molecules [76] that influenced the formation and stability of complexes between carriers and NA and enhanced interaction between dendriplexes and cells. Takahashi et al. [77] attached two dodecyl groups to PAMAM dendrimers and so enhanced transfection activity. Kono et al. [78] designed PAMAM dendrimers of 4th generation having on their surface hydrophobic amino acids phenylalanine or leucine, to provide for involvement of both electrostatic and hydrophobic interactions upon NA binding. Dendrimers with phenylalanine enhanced the efficiency of genetic material transfer in CV1 cells, but they were poorly soluble in aqueous solution. Joining leucine residues to dendrimer did not enhance transfer using such dendrimer, possibly due to low hydrophobicity of this amino acid.

Choi et al. [79] synthesized L-arginine PAMAM dendrimers with L-arginine groups attached to their surface. The ability for genetic material transfer in such dendrimers exceeded that in polylysine PAMAM dendrimers, while cytotoxicity level was far below that of PPI dendrimers. The same group checked the efficiency of genetic material transport in neuronal cells of newborn rats, which are especially resistant to non-viral pathways of genetic material delivery. Arginine PAMAM dendrimers exhibited a significantly higher level of genetic material transfer than commercially available agents and unmodified dendrimers. Kim et al. [80] synthesized arginine-conjugated PPI dendrimers and checked with HeLa and HEK293 cells the efficiency of genetic material transfer by these conjugates. As was shown, PPI–arginine conjugates were fourfold more efficient and less toxic for cells than the known carrier polyethyleneimine (PEI 125 kDa). Modification of dendrimer surface by guanidine [81] and investigation of DNA transfection by these dendrimers on cell lines HEK293T and COS-7 showed their lower efficiency and lower cytotoxicity compared to polyethyleneimine. Nam et al. [82] studied the possibility of obtaining PAMAM dendrimer esters with covalently attached arginine residues. Such conjugates not only exhibited higher efficiency than PEI 125 kDa in *in vitro* NA delivery into umbilical vein endothelial cells, but they were quickly destroyed after plasmid DNA delivery. After release of nucleic acids, they were hydrolyzed to nontoxic PAMAM-OH dendrimer and arginine. Choi et al. [83] suggested conjugation of PAMAM dendrimers with the known glucocorticosteroid dexamethasone. Such conjugates exhibited tenfold higher activity than PAMAM dendrimers and polyethyleneimine, especially in the presence of blood serum. Besides, total amount of conjugates that reached the cell nucleus was higher than for standard PAMAM dendrimers.

The efficiency of genetic material transfer by PAMAM dendrimers can be improved by their additional conjugation with cyclodextrins [84]. Cyclodextrins are cyclic saccharides containing a hydrophobic nucleus and hydrophilic surrounding, and they are used to improve drug stability, solubility, and bioavailability [85]. Polymer glycosylation is an efficient method of gene delivery to some cells [86]; therefore, the synergistic effect of dendrimers and cyclodextrin could be expected. In fact, addition of cyclodextrins to the dendrimer–NA complex improved distribution of the latter in aqueous conditions and more than 200-fold increased *in vitro* expression of chloramphenicol transacetylase (EC 2.3.1.28), which is indicative of efficient transfer of the gene for this protein. Especially high transfection efficiency and low *in vitro* cytotoxicity were shown by conjugates of low generation PPI dendrimers with cyclodextrins [87]. Arima et al. [88] synthesized conjugates of second generation PAMAM dendrimers with cyclodextrins and checked their efficiency. It was shown that modified dendrimers formed complexes with plasmid DNA, which was protected against degradation by DNase I (EC 3.1.21.1). Transfection efficiency of such dendriplexes increased 100-fold compared to unmodified dendriplexes. The use of cyclodextrins and dendrimers with mannose in surface groups resulted in a larger effect [89]. However, complete replacement of surface groups by mannose residues sharply decreased NA binding to the dendrimer. This shows that some of the positive charges on the dendrimer surface are necessary for complex formation with NA. In the case of mannose-containing dendrimers, especially efficient transfer of genetic material was observed for cells whose receptors were able to recognize mannose residues on the dendrimer surface. Tsutsumi et al. [90] analyzed efficiency of siRNA transfer by third generation PAMAM dendrimer conjugates with cyclodextrin and by such known carriers of genetic material as Lipofectamine™ 2000, TransFast™, and Lipofectin™. This conjugate was localized only in the cytoplasm, it was significantly less toxic than generally accepted carriers of genetic material, and it provided for constant and stable expression of the luciferase gene, which suggests its promise as an NA carrier. Kim et al. [91] synthesized a series of dendrimers from galactosyl residues and studied the possibility of genetic material transfection on their basis *in vitro* and *in vivo*. Optimal *in vivo* transfection activity in liver cells was achieved with dendrimers having three galactosyl residues and 16 dendrite branches.

Many researchers develop multilayer conjugates consisting of polymers and dendrimers. Zhang et al. [92] proposed conjugation of polylactide glycoside microspheres with PAMAM dendrimers. Such conjugates resembling dumbbells in shape had advantages from both components. First, cytotoxicity significantly decreased compared to standard PAMAM dendrimers and second, these conjugates provided for high efficiency of plasmid DNA transfer characteristic of PAMAM dendrimers. Fu et al. [93] tried PAMAM dendrimer immobilization on a solid carrier. Although the efficiency of transfer by such carriers was not very high, they concluded that this direction was promising for localized transfer on solid carriers. Li and Morcos [94] synthesized a dendrimer based on triazine with guanidine branches with conjugated antisense oligonucleotide Morpholino for its transfer into organs and tissues. Investigation of such dendrimer transfection in combination with plasmid DNA of green fluorescent protein showed *in vivo* efficiency of genetic material transfer in mice: the presence of oligonucleotide was observed both in the cell cytoplasm and nuclei of different organs and tissues. Shieh et al. [95] suggested the use of PAMAM dendrimer conjugates with porphyrin. These conjugates not only were able to transfer genetic material, but they exhibited phototoxic activity: upon illumination by light, the porphyrin generated free radicals and induced apoptosis in cells.

Cationic lipids are known carriers in gene therapy. In attempts to combine advantages of both cationic lipids and dendrimers, researchers created hybrids – lipid dendrimers [96]. Such amphiphilic dendrimers exhibited highly efficient delivery of genetic material [96-98]. Ewert et al. [99] synthesized dendrimers based on dioleyl phosphatidylcholine lipid and checked their applicability *in vitro*. The results of the investigations showed that these dendrimers were tenfold more efficient in transfer of plasmid DNA encoding luciferase gene in mouse lymphocytes than the known lipid carrier DOTAP. Simultaneously they showed five times lower cytotoxicity than DOTAP. Jones et al. [100] proposed for gene transfection new dendrimers based on combinations of cholesterol regions with spermine-functionalized dendrons.

A new and promising trend in dendrimer modification for delivery of genetic material is internal quaternization of their groups. Minko et al. [101, 102] synthesized dendrimers having (i) neutral surface of hydroxyl groups for lowering their cytotoxicity and (ii) internal charged cationic groups providing for NA binding. These quaternized dendrimers were compared with PAMAM-NH_2 dendrimers of the same generation. Analysis of their interaction with siRNA showed that quaternized dendrimers formed globular dendriplex with siRNA, whereas cationic PAMAM dendrimers formed nano-threads. Analysis of transfection using quaternized dendrimers *in vitro* on human ovary cancer cells has shown that, unlike standard dendrimers, quaternized dendrimers were observed in the cytoplasm and nucleus, which is indicative of high efficiency of siRNA transfer. Lee et al. [103, 104] synthesized internally quaternized PAMAM-OH dendrimers and studied their involvement in plasmid DNA delivery. Although these dendrimers formed stable dendriplexes, their efficiency in transfection was far below that of cationic PAMAM dendrimers. Probably the neutral surface of these dendrimers was the main reason of lowered transfection efficiency. Cationic dendrimers interacted with anionic surface and penetrated into cells easier than neutral or anionic dendrimers. However, positive charge of dendrimers, in turn, enhanced their toxicity. Tziveleka et al. [105] studied transfer of genes based on quaternized hyperbranched polyester polyols and found that the presence of positive charges on their surface does not enhance transfection efficiency with involvement of these dendrimers despite emergence in them of additional buffer capacity ("sponge effect"). They concluded that interaction of these dendrimers with the membrane follows a mechanism different from that for usual cationic dendrimers.

Interesting are investigations on genetic material transfection in mesenchymal stem cells using PAMAM dendrimers [106]. These dendrimers proved to be efficient carriers for plasmid DNA encoding the β-galactosidase gene.

Pasupathy et al. [107] opened a new direction and proposed the use of PAMAM dendrimers for gene delivery into plant cells. They delivered plasmid DNA encoding green fluorescent protein into lawn grass cells and observed transfection results by fluorescence microscopy. It was concluded on the basis of the results that the use of dendrimers for delivery of genetic material into plant cells is promising.

In conclusion, it would be desirable to mention a work by Hollins et al. [108]. They studied siRNA transfer to launch the mechanism of gene silencing using PAMAM dendrimers of the same generation but of different molecular structure. It was found that slight alterations in dendrimer structure resulted in almost tenfold difference in expression of epidermal growth factor against which siRNA was active. These results show that practically every siRNA, DNA, mRNA, and ODN needs its individual carrier, nontoxic and highly efficient. Therefore, the search for efficient synthetic vectors for gene therapy will remain active for a long time.

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