Surface Modifications of Cationic Polyamidoamine (PAMAM) Dendrimers

Katyonik Poliamidoamin (PAMAM) Dendrimerlerin Yüzey Modifikasyonları

Review Article

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ABSTRACT

PAMAM dendrimers are a new class of macromolecular polymers, with a highly branched three-dimensional structure that provides a high degree of surface functionality and versatility. These nanospherical polymers possess exclusive properties which make them potential carriers for drug and gene delivery. PAMAM dendrimers allow the precise control of size, shape and placement of functional groups that is desirable for many life science applications. However, positive charge at the surface renders cytotoxicity to the cationic PAMAM dendrimers, which limits their clinical usefulness. Modification of dendrimer surface groups is one of the methods available in order to reduce the toxicity and improve their biocompatibility. In this review, firstly a brief description is provided about the characteristics and structure of PAMAM dendrimers. Then, various modification routes with the aim of decreasing the cytotoxicity of PAMAM dendrimers are presented and discussed.

Key Words
PAMAM dendrimer, Surface modification, PEGylation, Acetylation

ÖZET

PAMAM dendrimerler, makromoleküler polimelerin yeni bir sınıfı olarak, çok dallı ve üç boyutlu yapıları aracılığıyla yüksek derecede yüzey fonksiyonelliği ve çok yönlülük sağlar. Bu nanoküresel polimerler, kendilerini ilaç taşıma ve gen aktarımı için potansiyel hale getiren seçkin özelliklere sahiptir. PAMAM dendrimerlerin, fonksiyonel grupların boyutu, şekli ve yerleşimi konusunda kesin kontrol sağlaması, birçok yaşam bilim uygulamaları tarafından kullanımlarının istenmesine neden olmuştur. Fakat, yüzeylerindeki pozitif yük, katyonik PAMAM dendrimerlere sitotoksite vererek, klinik kullanımlarını sınırlandırmaktadır. Dendrimer yüzey gruplarının modifikasyonu, toksisiteyi azaltmak ve biyouyumluluğu geliştirmek amacıyla uygulanan mevcut yöntemlerden biridir. Bu derlemede ilk olarak PAMAM dendrimerlerin özellikleri ve yapısı hakkında kısa bir açıklama sağlanmıştır. Daha sonrasında ise PAMAM dendrimerlerin sitotoksisitesini azaltılmak amaçlı çeşitli modifikasyon yöntemleri sunulmuş ve tartışılmıştır.

Anahtar Kelimeler
PAMAM dendrimer, Yüzey modifikasyonu, PEGilasyon, Asetilasyon

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INTRODUCTION

Polyamidoamine (PAMAM) dendrimers represent a novel class of biocompatible polymers which have been extensively used in biomedical applications. These nanospherical, well-designed branching polymers possess interior cavities and abundant terminal groups on the surface that can form stable complexes with drugs, plasmid DNA, oligonucleotides, and antibodies [1]. Therefore, these biocompatible, safe, and non-immunogenic polymers can function as highly efficient cationic polymer vectors for delivering genetic material and different drugs into cells. Additional advantages of PAMAM dendrimers include their acceptable biodegradation, ease of surface modification, their controlled drug release, and minimal non-specific blood–protein binding properties [2-6].

Structure of PAMAM dendrimers

In 1980s, synthesis of PAMAM dendrimer was reported by Tomalia and coworkers for the first time [7]. Basically, a PAMAM dendrimers possess three distinguishable architectural components: a core, interior layers (generations) consisting of repeating units radially attached to the core, and surface functional groups (Figure 1).

The growth of these highly organized and relatively monodisperse polymers emanates from a central core [8]. Selection of the core is of great importance, since it can affect the molecule and surface charge density [7]. Individual ''wedges'' or dendrons radiate from this central core, and each layer of concentric branching units is considered one complete generation (G) in the dendrimer

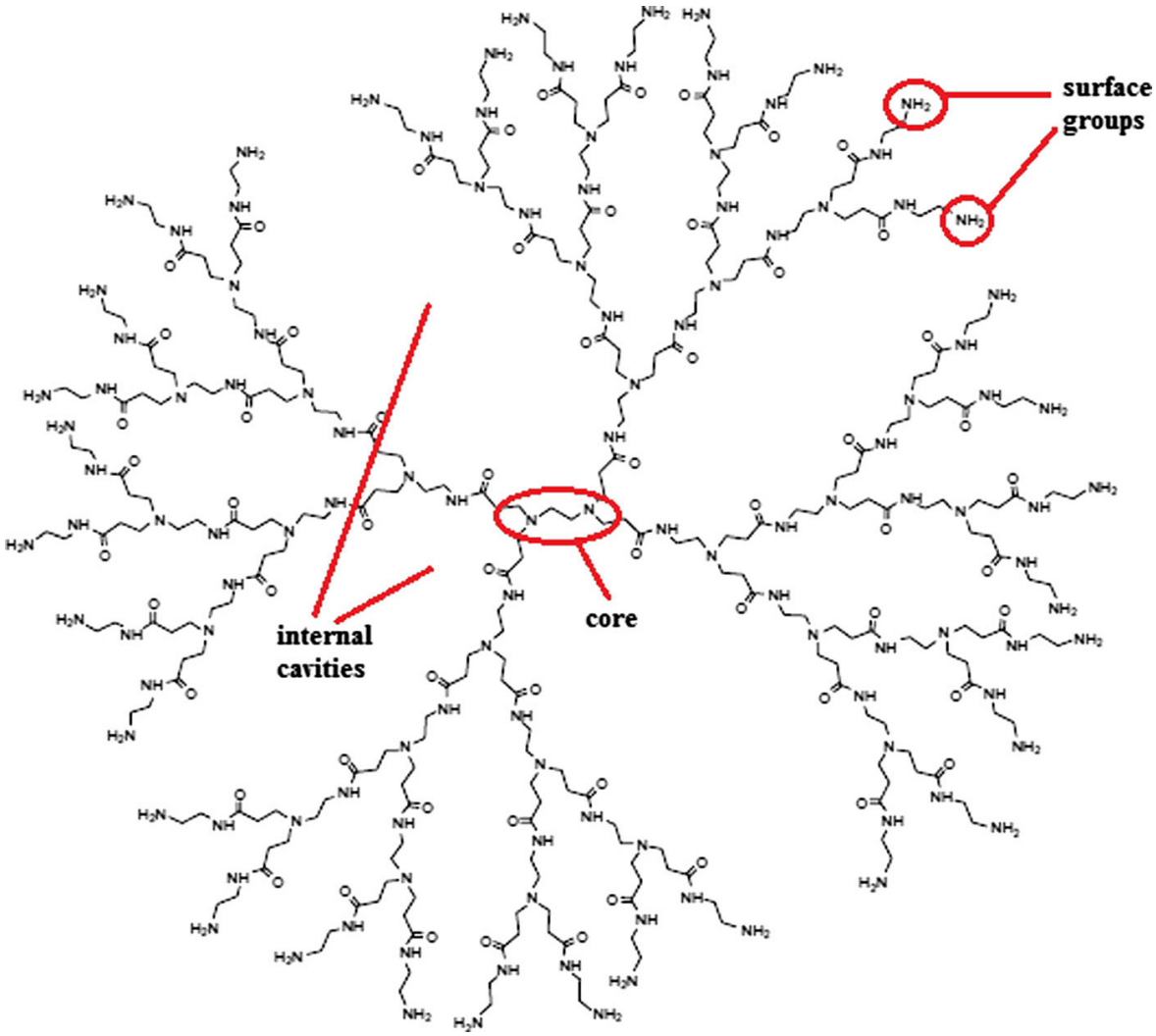

Figure 1. Structure of the PAMAM dendrimer.

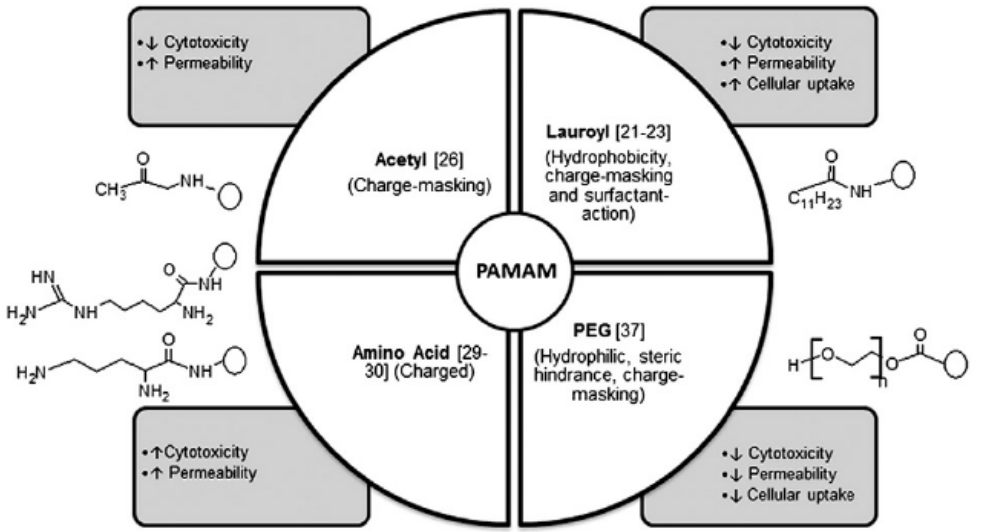

Figure 2. Effect of surface modifications on PAMAM cytotoxicity, transepithelial permeability and cellular uptake. Adapted from [16].

series and has a specific generation number. The size of PAMAM dendrimers ranges from 1 to 13 nm in diameter for generation 0 (G0) through generation 10 (G10). In other words, change of approximately 1 nm is seen in the molecular diameter as each generation varies. In detail, dendrimer diameter increases roughly linearly with generation, while the number of functional groups on the surface increases exponentially. An important consequence of this is that the distance between functional groups on the periphery of the dendrimer, and consequently the flexibility of peripheral groups, decreases with generation [9]. The polymer's shape is affected by the number of generations. Lower generation dendrimers (G0–G4) have a planar, elliptical shape, while at the higher generations (G5–G10), the densely packed branches induce the polymer to form a spherical conformation [4].

There are primary amine groups on the surface of PAMAM dendrimers and tertiary amine groups inside the molecule [9-12]. The primary amine groups play an essential role in binding DNA and inducing the cells to uptake the DNA, while the buried tertiary amino groups perform as proton sponge in endosomes that cause DNA release into the cytoplasm [13]. Furthermore, the hydrophobic empty spaces in the inner region of the PAMAM dendrimers of generations higher than four, can used for encapsulating different compounds or drug delivery applications. The literature shows that high aqueous solubility, unique architecture, high number of chemically versatile surface groups of PAMAM dendrimers are among the advantages which make them ideal carriers for the delivery of therapeutic agents including anticancer drugs [14]. The positive surface charge (-NH_2 groups) of cationic PAMAM dendrimers leads them to interact with negatively charged cell membranes and ends with the dendrimers permeation. However, such a positive surface charge renders cytotoxicity to the cationic PAMAM dendrimers, which limits their clinical usefulness. An alternative to overcome this substantial limitation is modification of the surface groups. In other words, dendrimers surface modifying is a method of engineering the surface of dendrimers with different functional groups to mask the cationic charge of dendrimers and convert them into biocompatible polymers. This has been supported either by neutralization of charge, for example, PEGylation, acetylation, folate, and peptide conjugation; or by introducing negative charge such as half generation dendrimers. Modification of dendrimers *via* the attachment of other molecules to dendrimers' surface groups seems to be the most prominent solution among the numerous options yet to be explored [15]. Figure 2 summarizes the effect of surface modification on cytotoxicity, cellular uptake and permeability.

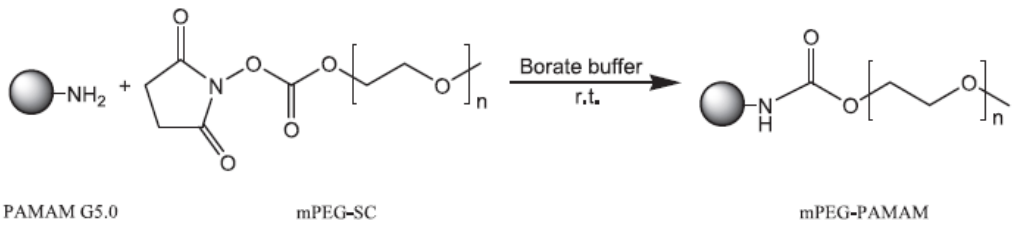

Figure 3. Synthesis of PEGylated PAMAM dendrimers. Adapted from [22].

1. Conjugation of PEG (PEGylation) to the periphery of PAMAM dendrimers

Polyethylene glycol (PEG) is a non-immunogenic, non-toxic, and highly water soluble polymer which has become an agent of particular interest in the design of dendrimer systems for pharmaceutical applications [3]. Covalent conjugation of PAMAM dendrimer with PEG is used as a strategy to overcome disadvantages due to surface amine groups. It was indicated that surface modification by uncharged groups such as PEG decreases the cytotoxicity of PAMAM dendrimer by charge masking of the primary amine groups. The incorporation of PEG chains into the PAMAM dendrimer structure can yield a number of biologically and pharmacokinetically desirable properties such as improved biocompatibility, increased structural stability, prolonged half-life, and enhanced water solubility of both drug and carrier system [17]. Engineering the surface groups of PAMAM dendrimers with PEG also alters their renal excretion, permeability and cellular uptake [17-19].

The effects of PAMAM dendrimer's PEGylation have been evaluated in drug delivery experiments. Bhadra et al. compared uncoated and PEGylated PAMAM dendrimers for delivery of anti-cancer drug, 5-fluorouracil. The results indicated that PEGylation of PAMAM dendrimers increased the drug-loading capacity, reduced drug release rate, and hemolytic toxicity. The use of such PEGylated dendrimeric systems has been suggested as nanoparticulate depot type of system for drug administration [20].

The influence of surface modification on the cytotoxicity of PAMAM dendrimers using Caco-2 cells was evaluated by Jevprasesphant and his coworkers. The modification was performed by conjugating either lauroyl chains or PEG to the surface of cationic PAMAM dendrimers. A marked decrease in the cytotoxicity of cationic PAMAM dendrimers was noticed when the surface was modified with the addition of six lauroyl or four PEG chains. This decrease in cytotoxicity is thought to be due to a reduction or shielding of the positive charge on the dendrimer surface by the attached chains [21].

Another investigation was performed in order to improve the biocompatibility of PAMAM dendrimers by conjugating them with PEG molecules (Figure 3). The IC_{50} values (concentration at which 50% of mitochondrial dehydrogenase activity was inhibited) of PEGylated dendrimers were 12–105 fold higher than those of PAMAM dendrimers. To investigate the influence of PEGylation on PAMAM-induced cytotoxicity, the intracellular responses, reactive oxygen species (ROS) content, mitochondrial membrane potential (MMP), and apoptosis were examined. The results indicated that conjugation with PEG could effectively reduce the PAMAM-induced cell apoptosis by attenuating the ROS production and inhibiting PAMAM-induced MMP collapse. Meanwhile, dendrimers conjugated with less PEG of lower molecular weight did not significantly change the endocytic properties. Dendrimers conjugated with more PEG of higher molecular weight were much less cytotoxic. This study provided a novel insight into the effects of PEGylation on the decrease of cytotoxicity at the molecular level [22].

Haba et al. prepared PEG-modified PAMAM dendrimers encapsulating gold nanoparticles (Au NPs) and investigated their stability and photothermal properties. The excellent colloidal stability, high heat-generating ability and their biocompatible surface confirmed that the PEGmodified dendrimers encapsulating Au NPs

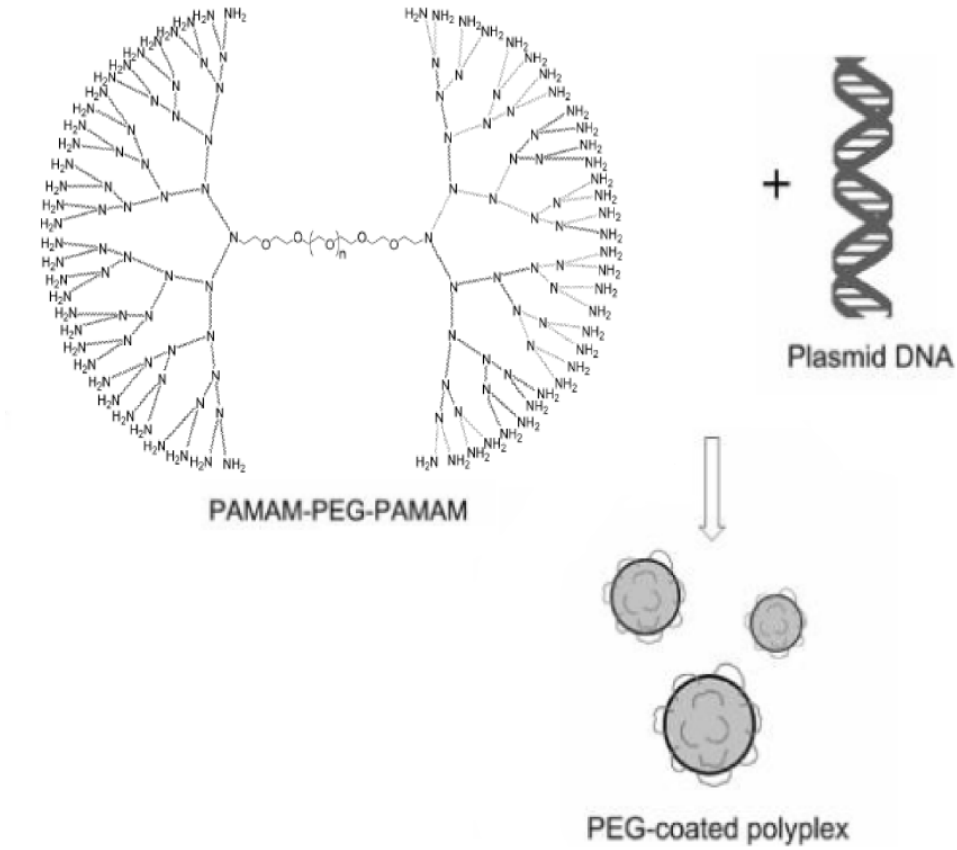

Figure 4. Schematic view of the formation of PEG-coated polyplexes. Adapted from [26].

are a promising tool for photothermal therapy and imaging [23].

The use of PEGylated PAMAM dendrimers for gene transfection has also been demonstrated. Qi et al. applied PEGylation in order to improve the characteristics of PAMAM dendrimer as gene delivery carriers and revealed that the degree of PEGylation is an important variable affecting the transfection efficiency of PEGylated PAMAM dendrimers. Compared with unconjugated PAMAM dendrimers, PEG conjugation significantly decreased the *in vitro* and *in vivo* cytotoxicities and hemolysis of dendrimers, especially at higher PEG molar ratios [24]. Luo et al. conjugated PEG to PAMAM dendrimer (generation 5) via a stable amide linkage and noticed its high efficiency in transfecting Chinese hamster ovarian cells [25].

Kim et al. developed cationic triblock copolymer, PAMAM-block-PEG-block-PAMAM, composed of a PEG core and two PAMAM substructures on both sides to enhance transfection efficiency. It was able to self-assemble with plasmid DNA forming a compact spherical polyplex (Figure 4), which showed greatly enhanced water solubility compared to the PAMAM dendrimer itself. The copolymer is proven to have little cytotoxicity in mammalian cells, low interaction with serum, and high transfection efficiency in 293 transformed human kidney cells [26].

Taken together, the above studies show that by addition PEG chains to the surface groups of PAMAM dendrimers, it is possible to change cytotoxicity, stability, permeability, and water solubility. PEGylation of dendrimers can also improve the circulation time of dendrimers in the body [27].

2. Acetylation of PAMAM dendrimers

Acetylation of the reactive end groups of PAMAM dendrimers has been another route to conceal the terminal amino groups of polycationic PAMAM dendrimers and minimize their toxicity. After

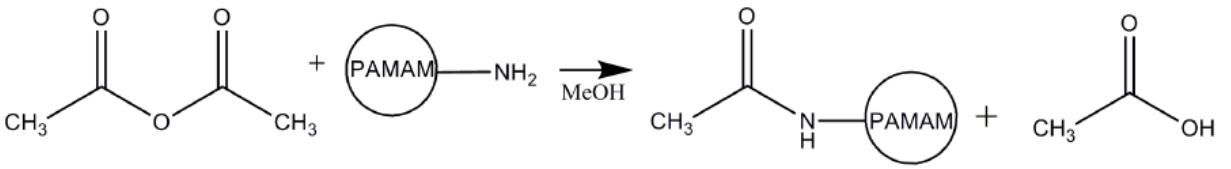

Figure 5. Acetylation of PAMAM dendrimers. Adapted from [30].

acetylation of PAMAM surfaces, they become more water-soluble, and this quality is vital for biomedical applications that require solubility in aqueous solutions [3]. The acetylated dendrimers unexpectedly exhibit smaller molecular size despite their increasing molecular weight, since acetylation of all primary amines considerably changes the structure of the dendrimer, which leads to a more compact structure than the non-acetylated PAMAM dendrimer [28].

In an investigation performed by Kolhatkar et al., the surface groups of amine-terminated PAMAM dendrimers were modified with acetyl groups. The earlier investigation of these authors indicated higher permeation of cationic PAMAM dendrimers across Caco-2 cells compared to their neutral or anionic counterparts. Consequently, as their next step they used acetylated PAMAM dendrimers in order to test the effect of this modification on cytotoxicity, permeability, and cellular uptake on the tested cell line. Cytotoxicity was reduced by more than 10-fold as the number of surface acetyl groups increased while maintaining permeability across the cell monolayers. Other factors that prompted the use of acetyl groups for surface functionalization were as follows: (1) an increasing interest in surface modified dendrimers for drug delivery; (2) ease of controlling the degree of acetylation by controlling stoichiometry; (3) mild reaction conditions required for acetylation and (4) increased solubility of acetylated dendrimers. Taken together, these studies suggest surface-modified PAMAM dendrimers for specific biomedical applications [29]. In addition Quintana et al. reported that acetylation can be used to reduce the non-specific binding associated with amine-terminated nanoparticles. A decrease in the number of positively charged surface groups may reduce the nonspecific interaction of acetylated PAMAM dendrimers with the negatively charged cell membrane. This, in turn, may result in a relatively higher concentration of free dendrimer at the apical side of the membrane, contributing to increased permeability through the paracellular route. These data are in agreement with studies by Wiwattanapattapee et al., who observed higher tissue uptake and lower serosal transfer rate for modified dendrimers, compared to non-modified ones. Moreover, PAMAM dendrimers carry a large number of internal tertiary amine groups, which can potentially interact with negatively charged cell membranes, enhancing permeability [3].

In addition, the effect of primary amine acetylation of the PAMAM dendrimer on siRNA delivery was analyzed. PAMAM dendrimers were reacted with acetic anhydride to obtain controlled extents of primary amine acetylation (Figure 5). Acetylated dendrimers were complexed with siRNA, and physical properties of the complexes were studied. Increasing amine acetylation resulted in reduced polymer cytotoxicity to U87 cells, as well as enhanced dissociation of dendrimer/siRNA complexes. Acetylation of dendrimers reduced the cellular delivery of siRNA which correlated with a reduction in the buffering capacity of dendrimers upon amine acetylation [30].

3. Addition of folic acid (folate) to the surface of PAMAM dendrimer

Folic acid (FA) is a stable and non-immunogenic ligand with a high affinity to the cell surface folate receptor (FR). The folate enters the cells via a receptor-mediated endocytosis providing cytosolic deposition. The strategy of active targeting via the FR can be successfully used for the site-specific delivery for drugs and prevent unwanted side effects [31].

In a research performed by Wang et al., PAMAM dendrimers were utilized to encapsulate an anticancer drug doxorubicin (DOX). In this

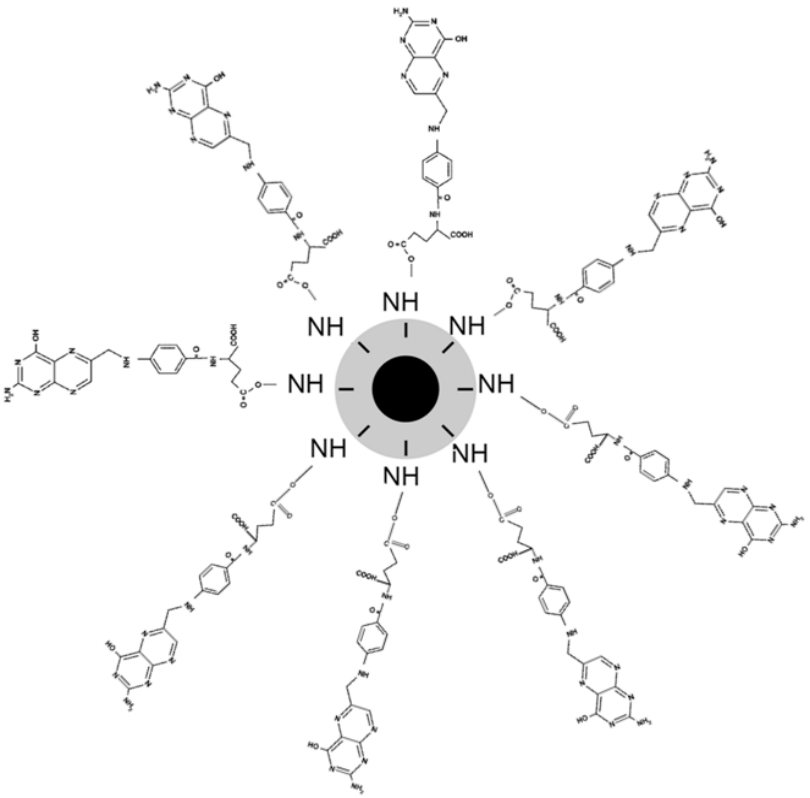

Figure 6. Chemical structure of folic acid–PEG4000–4G PAMAM dendrimers.

study, generation 5 (G5) PAMAM dendrimers were modified with FA and fluorescein isothiocyanate (FI) via covalent conjugation, and with remaining terminal amines being acetylated. The modified dendrimers were used as DOX delivery system to cancer cells overexpressing high-affinity FR. The results indicated that the synthesized stable complexes are able to specifically target to KB cells (human epithelial carcinoma cell line) with high-level FR and may serve as a general carriers for sustained release and targeted delivery of various anticancer drugs for a range of cancer therapeutics applications [32]. In another study, PAMAM dendrimers up to fourth generation were produced, characterized, and attached to FA directly or indirectly through PEG4000 as spacer. In other words, primary amines present on the dendritic surface were conjugated through FA and FA–PEG–NHS (N-hydroxysuccinimide) conjugates (Figure 6). Then, prepared dendritic conjugates were evaluated for the anticancer drug delivery potential using 5-FU in tumor-bearing mice. Results indicated that folate–PEG-dendrimer conjugate was significantly safe and effective in tumor targeting compared to a non-PEGylated formulation. Tailoring of dendrimers via PEG–FA reduced hemolytic toxicity, which led to a sustained drug release pattern as well as highest accumulation in the tumor area. Singh et al. suggested that because of the reduced toxicity and enhanced efficacy, FA–PEG-drug loaded G4PAMAM dendrimers can be used most suitably as a controlled and targeted drug delivery system for the delivery of anticancer drugs like 5-FU [27].

Another tumor targeted and pH-responsive drug release system that is based on FA conjugated to PEG-modified PAMAM dendrimers was synthesized and characterized. The anticancer drug DOX was conjugated to the dendrimers using hydrazine as the linker via hydrazone bonds, which are acid cleavable and can be used as an

ideal pH-responsive drug release system. The PEG moiety attached to the complexes provides the conjugates with excellent solubility and stability in an aqueous medium, which may increase the circulation time. The attached FA could target the conjugates to the FR. Of more importance, most DOX could be disassociated from the conjugates within the nuclei of tumor cells or regions nearby, because FA is an efficient target molecule. Taken together, these novel DOX-loaded conjugates have been shown as a successful system to enhance the effect of cancer therapy in the course of delivering drugs to the target sites [33]. Chandrasekar et al. synthesized a FA/G4-PAMAM dendrimer conjugate which was loaded with indomethacin, an anti-arthritic drug. The main aim of this study was to synthesize folate-dendrimer conjugates as suitable vehicle for carrying indomethacin and to determine its site specific targeting efficiency to inflammatory region in arthritic rats. Results demonstrated that indomethacin encapsulation or complexation efficiency enhanced with increasing folate content. The in vitro release profile showed a more controlled release of the drug with rising folate content. Pharmacokinetic and tissue distribution studies in arthritic rats had depicted preferential higher accumulation of indomethacin at the inflamed paw by the folate conjugates in contrast to dendrimer at equivalent dose. Among the conjugates, the formulation with 21 folate molecules was discovered to have good encapsulation efficiency, controlled release nature and highest targeting efficiency to the inflammatory region [30,33]. Overall, the literature shows that engineering of the dendritic surface with a targeting ligand such as folic acid (FA) can enhance the site-specific anticancer drug delivery [27].

4. Modification of PAMAM dendrimers with biotin molecules

Biotin is an essential micronutrient molecule used in several metabolic pathways throughout the body (*e.g.* fatty acid biosynthesis, gluconeogenesis) and its levels are high in rapidly proliferating cells such as cancer cells. Biotin has been shown to cross the blood-brain barrier (BBB), suggesting that biotinylated PAMAM dendrimers may also have the potential for delivering therapeutic drugs to the brain. Biotin-labeled dendrimers have been used in tumor and antibody targeting studies and biosensor design [34,35].

In order to investigate the various characteristics of the biotinylated PAMAM dendrimers, the biotin-PAMAM conjugates were synthesized and characterized. The microscopic studies indicated greater cancer cellular internalization of biotinylated dendrimers when compared with the non-modified PAMAM dendrimers. Moreover, cellular uptake and cytotoxicity of the biotinylated dendrimers increased with generation number of the dendrimer, which can be due to the greater cationic surface charge density that enabled increased interaction with receptors on the cell membrane. Higher cellular uptake of biotinylated dendrimers supports the previous data by Kitchens and coworkers in a Caco-2 cell permeability model. The data was encouraging in that the increased paracellular permeability of the higher generation PAMAM dendrimers combined with enhanced cancer cellular internalization mediated by biotin makes this carrier system very effective in cancer cell targeting and permeation across the tumor tissue [36]. Finally, the potential toxicity of biotinylated G4 PAMAM dendrimer conjugates were determined. The findings showed the higher levels of toxicity of G4 and G4-biotinylated PAMAM dendrimers than non-biotinylated counterparts. In other words, in contrast to naive PAMAM dendrimers, biotinylated ones are regarded to be more toxic, partially because of their potential mechanism of uptake across the blood–brain barrier (BBB), as biotin has proved to cross the BBB through carrier mediated endocytosis. Hence, biotinylated PAMAM dendrimer conjugates get into cells more than non-biotinylated PAMAM dendrimers, resulting to more cell death [37].

5. Conjugation of amino acids to the surface of PAMAM dendrimers

By end-capping the PAMAM dendrimers with amino acid motifs, enhanced solubility, reduced cytotoxicity, and reduced hemolytic toxicity could be achieved, while retaining the chemoselective reactivity and functional flexibility to conjugate drugs and/or imaging agents [38]. Up to now, basic amino acids such as lysine and ornithine were applied more for modification of PAMAM

dendrimers, since they have cationic charged groups that can contribute to the condensation of DNA and interaction with cellular membranes. It is suggested that the basic amino acid-conjugated PAMAM dendrimers could be utilized as promising gene delivery polymeric vectors for effective gene therapy [39].

5.1. Surface modification of PAMAM dendrimers with L-arginine

Recently, several arginine-rich peptides, known as cell penetration peptides (CPPs), such as transactivator of transcription (TAT) and oligoarginine have been intensively utilized for binding to PAMAM dendrimer to improve their outstanding transmembrane translocation ability [39-41]. The arginine residues are double positively charged in physiological conditions due to the presence of both a guanidinium group and a primary amine functionality. They can favorably interact with the negatively charged cell membrane-associated proteoglycans, thus intensifying membrane penetration. Therefore, a great deal of research has been performed on introducing arginine into various drug delivery vehicles such as PAMAM dendrimers to improve their transfection efficiency and enhance cellular uptake [42,43]. Such a strategy can be exploited to design arginine-bearing vectors for the delivery of siRNA therapeutics with the aim of increasing cellular uptake and consequently enhanced siRNA delivery and improved gene silencing [44]. In an investigation, a novel type of arginine-rich dendrimer was designed by Chio et al., with a structure based on PAMAM dendrimer. The primary amines located on the surface of PAMAM were conjugated with arginines or lysines, which were called PAMAM-Arg and PAMAM-Lys, respectively. PAMAM-Arg showed enhanced gene expression in HepG2 and Neuro 2A cell lines and for primary rat vascular smooth muscle cells in comparison with native PAMAM and PAMAM-Lys. This constitutes a subnanosized three dimensional and multivalent arginine multimer, which possesses the potential to be an efficient gene carrier. It was shown that the outstanding transfection efficiency with relatively low cytotoxicity and ease of preparation would make PAMAM-Arg a promising nonviral vector for both *in vitro* and *in vivo* use. Potentially, PAMAM-Arg could be used as a dendritic carrier molecule and could encapsulate or entangle cargo molecules such as small molecules, peptides, proteins, oligonucleotides, and plasmids that are deficient in cell-penetrating or plasma membrane crossing capability [45].

In another study, Liu et al. conjugated arginine components onto the surface of triethanolamine (TEA)-core PAMAM dendrimer (G4) with the aim of combining and harnessing the unique siRNA delivery properties of the structurally flexible dendrimers and the cell-penetrating advantages of arginine-rich motifs to enhance the cell membrane penetration and foster delivery efficiency (Figure 7). The results demonstrated that the dendrimer G4-Arg was bestowed with a superior capacity to form stable dendriplexes with siRNA and enhance cell uptake, resulting in significantly magnified gene silencing by comparison with its amine-terminated dendrimer counterpart G4. Moreover, the G4-Arg delivery system displayed no

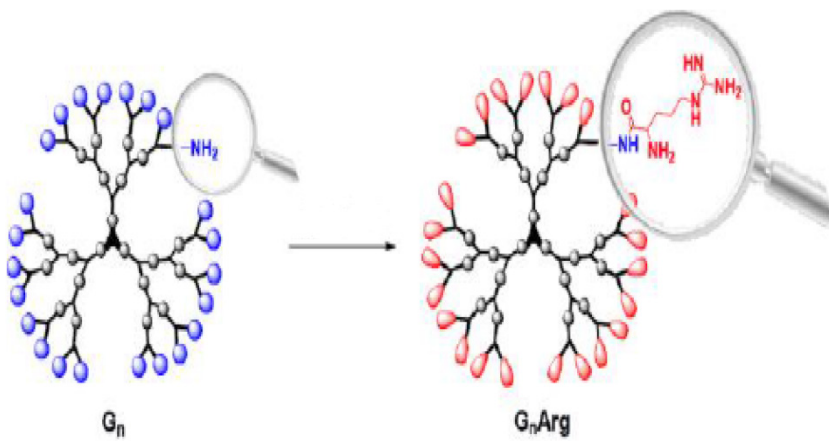

Figure 7. Synthesis of arginine-terminated dendrimers using amine-terminated TEA-core PAMAM dendrimers. Adapted from [44].

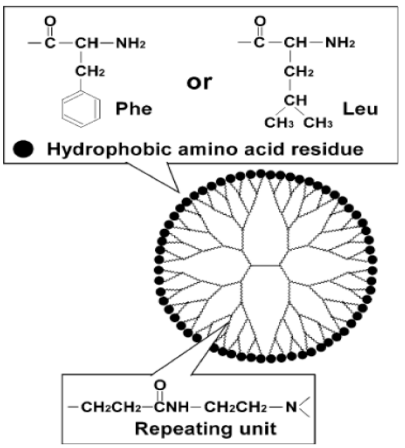

Figure 8. Structure of phenylalanine- or leucine-modified PAMAM G4 dendrimer. Adapted from [48].

discernible toxicity and was able to deliver siRNA in prostate cancer models both *in vitro* and *in vivo*, producing significant gene silencing and potent anticancer activity and holds great potential for further therapeutic applications [44].

The results of another investigation which evaluates arginine-modified PAMAM dendrimers for gene delivery suggested that attachment of arginine to PAMAM dendrimers through a biodegradable ester bond has at least two advantages. First, the introduced arginine affects polyplex formation and penetration into the cell membrane and nucleus. Second, the biodegradability of polymers affects the efficiency of intracellular disassembly for oligonucleotides and siRNAs, and thus cell viability [46].

5.2. Ornithine-conjugated PAMAM dendrimers

Ornithine is an amino acid that plays a role in the urea cycle. In a study, ornithine-conjugated PAMAM dendrimers (PAMAMG4-ORN) were prepared. PAMAM-ORN dendrimers have also shown high transfection efficiency as compared to parent PAMAM dendrimers in HEK 293T, GM7373 and NCI H157G cell lines. Though the exact basis for the high transfection efficiency of PAMAM-ORN dendrimers is not known, one possibility is that conjugation with ornithine increases the surface charge density of the dendrimer resulting in enhanced adsorptive endocytosis [2]. In a study performed by Pisal *et al*., the permeability across Caco-2 cell monolayers, and cytotoxicity of PAMAM G4 dendrimers conjugated with arginine and ornithine, was examined. The obtained data indicated that the surface modified dendrimers are transported across epithelial monolayers significantly faster than their unmodified counterparts. Toxicity tests revealed that PAMAM-arginine dendrimers have been found to be slightly toxic than ornithine-PAMAMs and unmodified PAMAM dendrimers. Whether it is reasonable to attribute the toxicity of PAMAM-arginine to elevated permeability remains to be established [47].

5.3. Attachment of hydrophobic amino acid residues (phenylalanine and leucine) to the periphery of PAMAM dendrimers

In a study performed by Kono et al., PAMAM dendrimers (G4) were designed having hydrophobic amino acid, phenylalanine or leucine residues at the dendrimer chain ends (Figure 8). The conjugates achieve cell transfection by synergistic actions of hydrophobic interactions and the proton sponge effect. The attachment of phenylalanine greatly improved the transfection activity of the parent dendrimer. However, transfection activity was not increased when leucine was attached to the dendrimer, probably because of the relatively lower hydrophobicity of this amino acid [48].

6. Surface modification of PAMAM dendrimers with lauroyl chains

The fatty acid conjugation such as lauroyl chains to PAMAM dendrimers was suggested as an efficient method in order to reduce toxicity and improve permeability. The reduction in cytotoxicity can be explained by shielding of the positively charged amine groups to interact with negatively charged cell surface proteins [16]. Additionally, it has shown that modification with lauroyl chains enhances the internalization kinetics of modified PAMAM dendrimer into human intestinal epithelial cells and may have the potential for improving drug delivery via the oral route [49].

7. Dimethyl itaconate as a PAMAM dendrimer modifier agent

In a study performed by Ciolkowski et al., the amine surface groups of a G4 PAMAM-NH2 dendrimer were transformed into pyrrolidone

derivatives by means of reaction with dimethyl itaconate. The results demonstrated that modified PAMAM-pyrrolidone dendrimer revealed no hemolytic activity, minor cytotoxicity against mouse neuroblastoma cell line and lowered affinity to human serum albumin. It has also suggested that such modified dendrimers can be used in nanomedical applications, as they possess the advantage of dendrimers' spatial structure and simultaneously possess reduced toxic activity [50].

Apart from the cell membrane nanopore formation, the mechanism of cytotoxicity of unmodified PAMAM dendrimers is believed to be related to the generation of reactive oxygen species (ROS) and damage of the mitochondria. Therefore, the authors decided to perform additional study to examine the ability of PAMAM-pyrrolidone dendrimer to induce reactive oxygen species (ROS) generation, changes in mitochondrial membrane potential and apoptotic cell death. Chinese hamsters fibroblasts (B14), embryonic mouse hippocampal cells (mHippoE-18) and rat liver derived cells (BRL-3A) were used to investigate the influence of pyrrolidone dendrimer. The analyzed dendrimer showed only minor toxicity and no ability to induce apoptosis. The most important finding wass the lack of influence of the PAMAM-pyrrolidone dendrimer on intracellular ROS level and mitochondrial membrane potential [51].

CONCLUSION

Despite the high efficiency of PAMAM dendrimers in the pharmaceutical applications, there is conflicting evidence regarding their biological safety, since cationic PAMAM dendrimers have been shown to possess concentration and generation-dependent cytotoxicity and hemolytic activity, properties that are associated with their terminal amine groups. Taken together, the results above show that a worthwhile strategy to overcome this drawback is modifying PAMAM dendrimers at their periphery which this, in turn, could minimize the cytotoxicity of the PAMAM dendrimers and make much more useful for specific biomedical applications. Some of the various supporting surface engineering strategies for PAMAM dendrimers are PEGylation, acetylation, folate, and peptide conjugation. The development of new methods in the modification of the PAMAM dendrimers and the many beneficial attributes of these dendrimers described in this review are a strong impetus for considering these tree-like macromolecules as the preferred polymeric nanocarrier to act in crucial biomedical applications such as drug delivery, gene transfection, and other strategies for the treatment of human disease. Also as research progresses, there will be opportunity for emerging newer and more features of PAMAM dendrimers especially in pharmaceutical and biomedical systems.

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