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REVIEW

Fabrication of Low-Generation Dendrimers into Nanostructures for Efficient and Nontoxic Gene Delivery

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Abstract Dendrimers with well-defined molecular structure and high monodispersity have gained tremendous interest in gene delivery. However, current gene carriers based on dendrimers are either not effective or are too toxic on the transfected cells. The efficacy and cytotoxicity of dendrimers are strongly correlated with their molecular weight or generation. High-generation dendrimers are reported with relatively high transfection efficacy but serious cytotoxicity due to the excess positive charges on the polymers, while low-generation dendrimers with minimal toxicity have poor polyplex stability and thus weak transfection efficacy. To break up the correlation between efficacy and toxicity, low-generation dendrimers were fabricated into various nanostructures by several strategies to improve their gene-binding capacity, polyplex stability, and transfection efficacy without inducing additional toxicity. In this review article, we will highlight recent advances in the development of assembled dendrimer nanostructures for efficient and non-toxic gene delivery. Specifically, the principles and strategies in the fabrication of dendrimer nanostructures are intensively reviewed.

Keywords Dendrimer · Gene delivery · Cytotoxicity · Transfection efficacy · Supramolecular chemistry

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1 Introduction

Dendrimers are hyperbranched macromolecules with well-defined molecular structures, high monodispersity, and nanoscale size [1–3]. They are synthesized in a step-wise manner around a core [4]. During the synthesis, each successive reaction step leads to an additional generation of branching and the number of repeated steps is defined as dendrimer generation (denoted as G) [5]. Generally, dendrimers with $G < 4$ and $G \geq 4$ are termed low- and high-generation dendrimers, respectively. Since the early 1990s, cationic dendrimers were widely used as non-viral vectors for gene delivery [6–10]. Generally, cationic dendrimers bind nucleic acids such as DNA and siRNA via ionic interactions, forming positively charged polyplexes. The polyplexes protect the nucleic acids from enzymatic degradation and favor their internalization by the target cells [11]. However, the polyplexes formed by ionic interactions are easily destabilized by salts, polyanionic proteins, and phospholipids abundant in biological systems [12]. As a result, low-generation dendrimers usually exhibit poor transfection efficacy, and high-generation ones with relatively high molecular weights and excess positive charges are used to increase the polyplex stability towards polyanionic molecules [6]. Though these dendrimers have increased transfection efficacy compared to low-generation ones, the excess of positive charges on the polyplexes usually lead to serious toxicity on the transfected cells (Fig. 1) [13–16]. The cytotoxicity of polymers is reported to increase with molecular weight and charge density [17–19]. Based on these rationales, there is an urgent need to break up the transfection efficacy-cytotoxicity correlation for cationic dendrimers in gene delivery [20]. Recently, low-generation dendrimers were fabricated into different nanostructures to dissolve the dilemma of balancing cytotoxicity and transfection efficiency for cationic dendrimers [21–25]. These fabricated nanostructures with limited positive charges can form stable polyplexes with DNA and siRNA in cell culture media, and further disassemble/degrade into low molecular weight species after cellular uptake [20, 21]. In this study, we will discuss the strategies in the fabrication of low-generation dendrimers for efficient and non-toxic gene delivery.

2 Assembling of Low-Generation Dendrimers into Nanostructures

Low-generation dendrimers can be fabricated into nanostructures via a supramolecular strategy. The dendrimers could be associated with each other in aqueous solutions via ionic, hydrogen-bonding, hydrophobic, or fluorophilic interactions [20, 23, 24, 26, 27]. When a G2 polyamidoamine (PAMAM) dendrimer was conjugated with an average number of seven phenylboronic acid moieties on its surface, the yielding zwitterionic dendrimer was clustered into nanoparticles around 100 nm via ionic interactions between phenylboronic acid and amine groups on the dendrimer surface (Fig. 2) [20]. The clustered G2 dendrimers exhibited superior DNA and siRNA delivery efficacy to high-generation dendrimers (such as G5), and comparable efficacy to Lipofectamine 2000. More importantly, the assembled

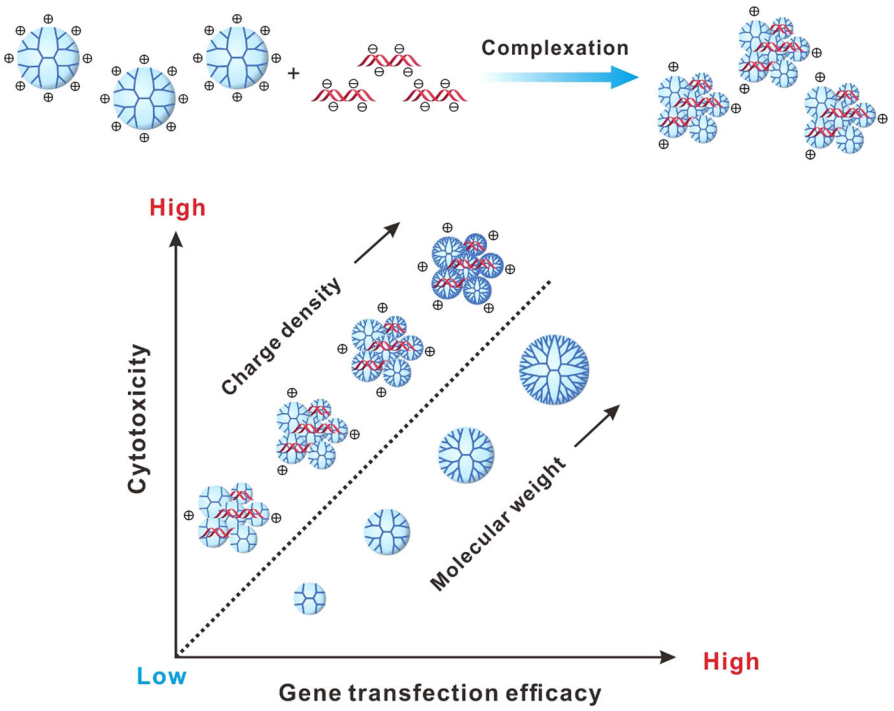

Fig. 1 Transfection efficacy and cytotoxicity of cationic dendrimers. Cationic dendrimers form polyplexes with nucleic acids via ionic interactions. The transfection efficacy and cytotoxicity of dendrimers are strongly correlated with the dendrimer generation and polyplex charge density

nanoparticles could quickly disassemble into low-molecular dendrimers when entrapped in acidic vesicles, leading to significantly decreased toxicity on the transfected cells. The analogue materials without the ability of self-assembly before complexation with DNA showed extremely low transfection efficacy. Both the phenyl and boronic acid groups play essential roles in the assembly and gene-delivery processes. Competitive binding of boronic acid moieties on G2 dendrimer with diols inhibited the formation of assembled nanostructures, and significantly down-regulated its transfection efficacy [20].

Similarly, hydrogen-bonding interaction can be adapted to fabricate nanostructures based on low molecular weight dendrimers. For example, melamine-modified G3 PAMAM dendrimer assembled into nanoparticles around 100 nm in aqueous solution when added with cyanuric acid (Fig. 3) [27, 28]. The formation of nanoparticles was driven by specific hydrogen-bond recognition between melamine and cyanuric acid. Melamine-modified G3 dendrimer alone formed loose polyplexes with plasmid DNA, which could be easily destabilized by polyanionic molecules such as heparin. In comparison, the presence of cyanuric acid significantly increased the polyplex stability via intermolecular hydrogen-bond recognition, and the strengthened polyplex exhibited much improved cellular uptake and transfection efficacy. Furthermore, the addition of cyanuric acid did not induce additional

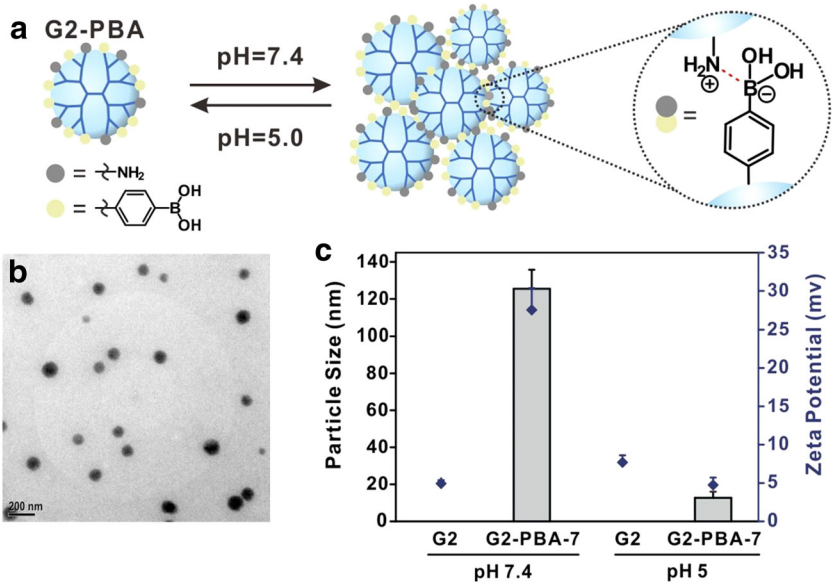

Fig. 2 **a** Self-assembly of phenylboronic acid-modified G2 dendrimer (G2-PBA-7) at pH 7.4 and disassembly of the nanoaggregates at pH 5.0. **b** TEM image of the assembled G2 dendrimers. **c** Hydrodynamic size of the intact G2 dendrimer and self-assembled G2-PBA-7 at pH 7.4 and 5.0, respectively. Reproduced from Ref. [20] with permission from Wiley 2016

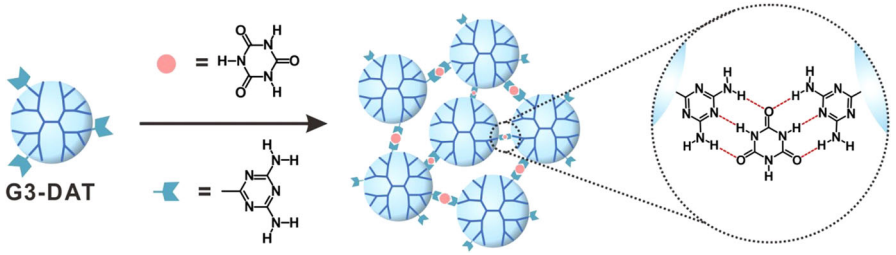

Fig. 3 Fabrication of low-generation dendrimers into nanoaggregates via the complementary hydrogen–bond interactions between cyanuric acid and melamine. The melamine moieties were modified on the surface of low-generation dendrimers (G3-DAT) via a facile reaction

toxicity on the transfected cells. This facile hydrogen-bond recognition strategy is beneficial for low molecular weight polymers in gene delivery.

When a low molecular weight dendron was conjugated with one or two aliphatic chains at the focal point of dendron, the generated amphiphilic polymers could self-assemble into nanostructures via hydrophobic interactions [22, 23, 29–37]. The aliphatic chain could be conjugated to the dendron by in situ synthesis, click chemistry, or host–guest interaction (Fig. 4) [22, 31, 38]. The aliphatic chain in the polymer controls the number of dendrons in each assembled nanostructure, as well as the diameter and surface charge density of the nanostructure, while the dendron generation influences its DNA binding and endosomal escape capacity [31, 39]. An

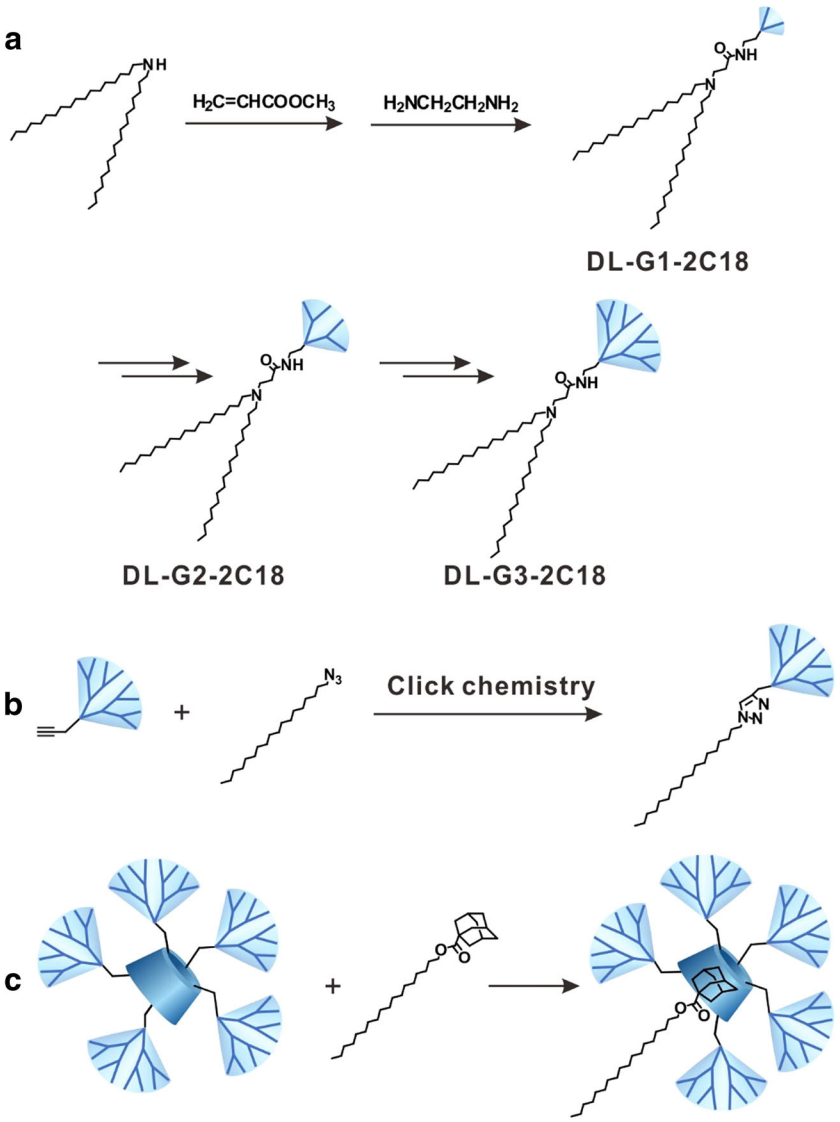

Fig. 4 Synthesis of lipid-bearing dendrons by in situ synthesis (**a**), click chemistry (**b**), or host–guest interaction (**c**)

amphiphilic polymer bearing a hydrophobic alkyl chain with 18 carbon atoms (C18) and a hydrophilic G1 PAMAM dendron with eight primary amines was capable of assembling into nanomicelles (Fig. 5a2) [22]. The assembled micelle had a similar structure to traditional high-generation dendrimers, and could efficiently deliver DNA and siRNA into various cell lines with minimal toxicity (Fig. 5b) [40]. Similarly, an amphiphilic material consisted of two C18 aliphatic chains and a G2 PAMAM dendron assembled into nanovesicles before siRNA complexation, and

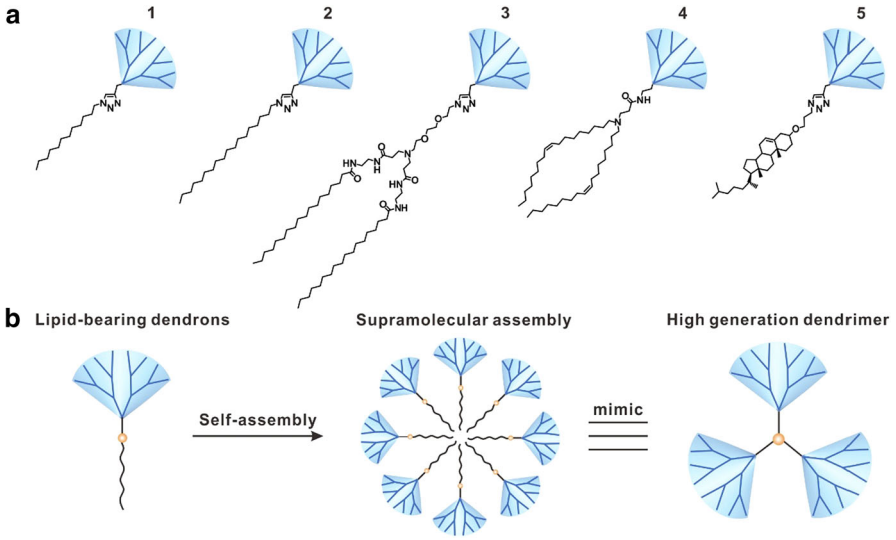

Fig. 5 The lipid-bearing dendrons designed for gene delivery (**a**). Self-assembly of the lipid-bearing low-generation dendrons into supramolecular structures to mimic the function of high-generation dendrimers in gene delivery (**b**). Reproduced from Ref. [40] with permission from Wiley 2016

achieved high efficacy in gene silencing (Fig. 5a3) [23]. Coarse-grained molecular dynamics simulation was further used to describe how the amphiphilic polymer self-assembled into nanostructures, and to analyze the optimal chain length and dendron generation on siRNA delivery [41]. According to the simulation results, the amphiphilic material with a C18 aliphatic chain and a G2 PAMAM dendron showed the best performance in siRNA binding, which is in accordance with the gene-silencing results reported by Peng et al. [22, 40]. Besides the alkyl chain length and dendron generation, the structure of lipid conjugated to dendron also plays an essential role in gene transfection [42–44]. G1 PAMAM dendron conjugated with two unsaturated C18 chains showed significantly increased gene-transfection efficacy compared to the analogue material containing two saturated C18 alkyls, probably due to better DNA packaging and smaller polyplex size (Fig. 5a4) [42]. This result might also be explained by distinct endosomal membrane disruption behaviors [45].

Besides aliphatic chains, hydrophobic ligands such as cholesterol could be anchored to low molecular weight dendrons to generate amphiphilic polymers for efficient gene delivery. Cholesterol is a naturally occurring building block that should be well tolerated in biological systems [46, 47]. The cholesterol-cored dendron with spermine as terminal groups could self-assemble into nanostructures, and disrupt the endosomal membranes via interactions between cholesterol and membrane phospholipids [46]. When cholesterol was conjugated to a degradable aliphatic ester-based dendron with a low molecular weight, the self-assembly will significantly enhance the DNA binding and cellular uptake of unmodified dendrons (Fig. 5a5) [48]. The modification of alkyl chains, cholesterol, or dexamethasone on

the surface of low-generation dendrimers also generated amphiphilic materials, which was capable of assembling into various nanostructures [47, 49]. These assembled nanostructures exhibited superior transfection efficacy to non-assembled analogues and better biocompatibility than high-generation dendrimers.

The assembled structures for alkyl chain- and cholesterol-modified dendrons or dendrimers were stable in aqueous solutions, but could be destabilized by phospholipids when penetrating across the cell membranes. To resolve this problem, we can introduce fluoroalkyls to replace the alkyl chain or cholesterol in the supramolecular amphiphiles. Fluoroalkyl chains are both hydrophobic and lipophobic, and the fluorocarbon chains can associate with each other through a fluorophilic effect in both hydrophilic and hydrophobic environments [50]. Percec et al. found that replacement of lipids on a polymer with fluoroalkyls caused drastic changes in the assembled structures [51], and the fluorine made a difference in the self-assembly process [52]. These features are promising for the fabrication of low molecular weight dendrimers into nanostructures via the fluorophilic effect. G1 and G2 PAMAM dendrimers modified with heptafluorobutyric acid molecules assembled into uniform nanoparticles (70−100 nm) with sizes similar to viruses (Fig. 6). The assembled structures could be modulated by tailoring the average number of conjugated heptafluorobutyric acid on the dendrimer surface [24]. Increasing the

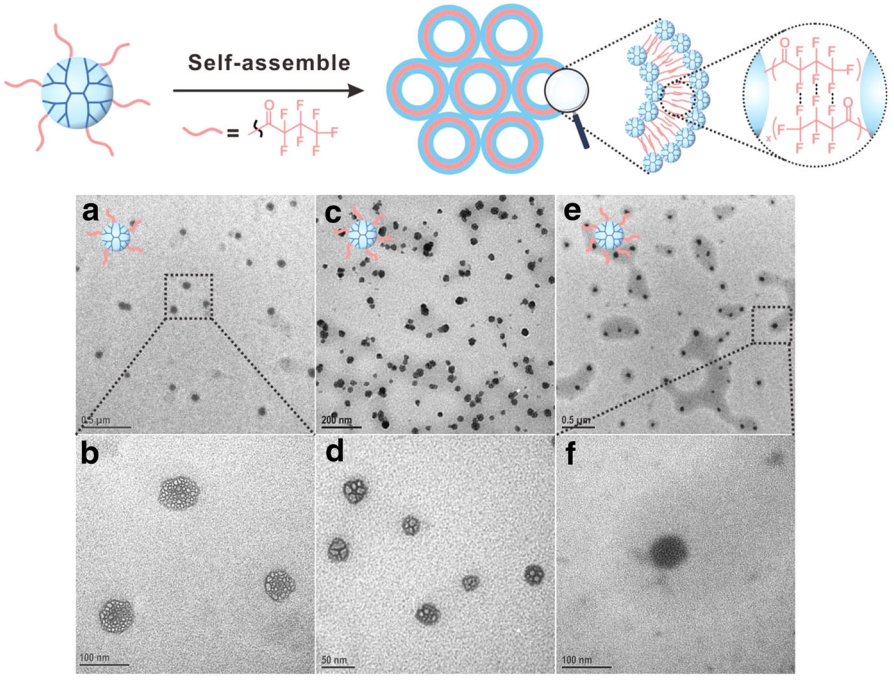

Fig. 6 Self-assembly of low-generation dendrimers modified with fluoroalkyls in aqueous solutions for efficient gene delivery (**a**). The assembled structures of G1 PAMAM dendrimer modified with an average number of 5, 6, and 7 heptafluorobutyric acids, respectively (**b**). Reproduced from Ref. [24] with permission from Wiley 2015

conjugation number on the dendrimer surface achieved high transfection efficacy at extremely low nitrogen-to-phosphorus ratios. The most efficient assembly showed high transfection efficacy on cells at an ultra-low DNA dose of 20 ng, maintained high efficacy even in the presence of 50% serum, and effectively transfected 3D spheroids and solid tumors in vivo [24, 53]. Since the synthesized materials have low molecular weight and charge density, they showed minimal toxicity on the transfected cells [54]. Besides self-assembly and polyplex stability, fluoroalkyls exhibited other advantages compared to alkyls in gene delivery mediated by amphiphilic dendrimers. First, fluoroalkyls are much more hydrophobic than alkyls with the same number of carbon atoms. As discussed in the section of amphiphilic dendrimers bearing alkyl chains, a long aliphatic chain with 12–18 carbon atoms was usually used to construct efficient amphiphiles. However, only four carbon atoms (heptafluorobutyric acid) were needed to achieve efficient gene delivery for fluoroaklyl-modified dendrimers. In addition, fluorocarbon chains can improve the transfection efficacy of cationic polymers by a "fluorous effect" (increased serum resistance, cellular uptake, endosomal escape, and easier intracellular DNA release) [54–59]. Currently, nearly 25% of the marketed drugs contain one or more fluorine atoms. These fluorine-containing drugs such as sorafenib and 5-fluorouracil could be loaded within the assembled fluorodendrimers via fluorophilic interactions [60]. The drug loading did not influence the gene-transfection efficacy of fluorodendrimers. As a result, these fluorophilic effect-driven assemblies enable the co-delivery of therapeutic drugs and genes for synergistic cancer therapy.

3 Anchoring Low-Generation Dendrimers onto Nanoparticles, Proteins, or Polymers

Low-generation dendrimers or dendrons could be grafted on biocompatible nanoparticles, proteins, or polymers via stimuli-responsive linkages to "temporarily" generate hybrid materials with relatively high charge density for efficient and non-toxic gene delivery. Lipoic acid-cored low-generation peptide dendrons were conjugated to the surface of inorganic nanoparticles such as gold, iron oxide, and quantum dots via covalent linkages (Fig. 7a) [25]. This strategy dramatically increased the gene-transfection efficiency of low molecular weight dendrons by approximately 50,000-fold. The linkage such as gold-thiol bond between the dendrons and inorganic nanoparticle was cleavable in the presence of glutathione after endocytosis [61]. As a result, minimal toxicity of the hybrid materials was observed during gene transfection. In addition, the inorganic nanoparticle endows the hybrid materials with new functions in gene delivery, e.g., gold nanoparticles for X-ray computed tomography or combined photothermal therapy, iron oxide nanoparticles for magnetic resonance imaging, and quantum dots (QDs) for fluorescence imaging [25]. The inherent fluorescence could be used to monitor intracellular pathway of the hybrid material as well as the protein expression. Similarly, gold nanoparticles engineered with low molecular weight dendrons showed high efficacy in siRNA delivery. The hybrid material suppressed the expression of a target gene by 50% with minimal toxicity. The dendronized gold

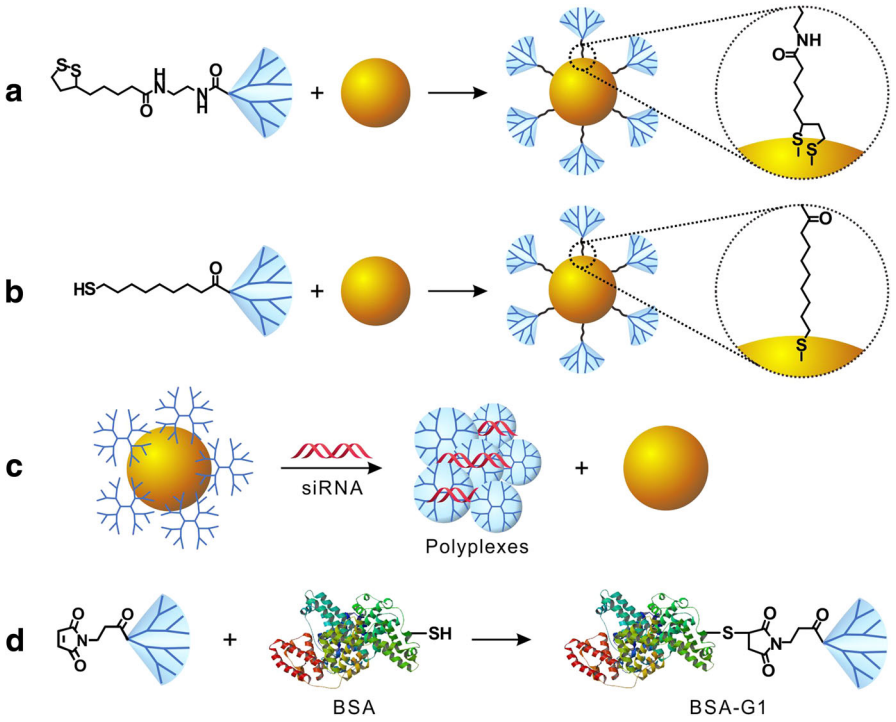

Fig. 7 Anchoring low molecular weight dendrons onto the surface of gold nanoparticles via Au–S linkage (**a**, **b**), Au-NH_2 complexation (**c**), or the surface of protein via click chemistry (**d**)

nanoparticles possessed high siRNA binding capability like high molecular weight polymers, while minimizing toxicity through the use of non-toxic core functionality (Fig. 7b) [62]. In a separate study, G2 PAMAM dendrimer was conjugated to mesoporous silica nanosphere for efficient gene delivery [63]. The yielded hybrid material could bind plasmid DNA to form stable polyplexes and efficiently deliver the DNA into hard-to-transfect cells such as neural glia cells. Since the mesoporous silica nanoparticles could be loaded with various drugs or proteins [64–66], the synthesized hybrid material allows the co-delivery of therapeutic genes and drugs for synergistic therapy. Though these low-generation dendrimer-coated silica nanoparticles showed efficient cellular uptake, their gene-transfection efficacy was not reported, and the cytotoxicity issue should be a problem for this hybrid material because the low molecular weight dendrimer was conjugated on nanoparticle surface via a non-degradable linkage.

Dendrimers could serve as ideal templates to synthesize dendrimer-encapsulated or dendrimer-stabilized nanoparticles such as gold and platinum nanoparticles [67–70]. When a G3 polypropylenimine (PPI) dendrimer was used as a template to synthesize dendrimer-stabilized gold nanoparticles, the gold nanoparticle in the hybrid material facilitated the low-generation dendrimer on its surface to package siRNA into compact polyplexes (Fig. 7c) [71]. The G3 PPI-stabilized gold nanoparticle showed much higher gene silencing efficacy compared to G3 PPI

dendrimer alone, and was even more efficient than a G5 PPI dendrimer. Interestingly, the gold nanoparticles in the hybrid materials were not included in the yielding polyplexes with siRNA, and this property made it possible to eliminate the potential cytotoxicity issues associated with gold nanoparticles, which were selectively removed from the polyplexes before cell endocytosis [71].

Low-generation dendrons could also be attached to the surface of proteins in a site-specific manner to generate dendron-conjugated proteins (Fig. 7d) [72]. The number of dendrons and the anchoring site on protein surface could be precisely controlled. For example, an *N*-maleimido-cored G1 dendron terminated with spermine was conjugated to thiol groups on the protein surface (e.g., Cys-34 on bovine serum albumin or a genetically engineered cysteine mutant of Class II hydrophobin) via biorthogonal chemistry. The dendron-anchored protein showed high affinity towards plasmid DNA, and exhibited significantly improved gene-transfection efficacy when delivering a plasmid encoding β-galactosidase [72]. The presence of proteins in the hybrid materials did not bring additional toxicity to the low molecular weight dendron on the treated cells.

By grafting low-generation dendrimers or dendrons onto biocompatible polymers such as chitosan, amylose, and polyrotaxane, the transfection efficacy of dendrimer and dendron could be significantly increased due to improved DNA binding and polyplex stability [73–75]. G2 and G3 PAMAM dendrons were conjugated to the

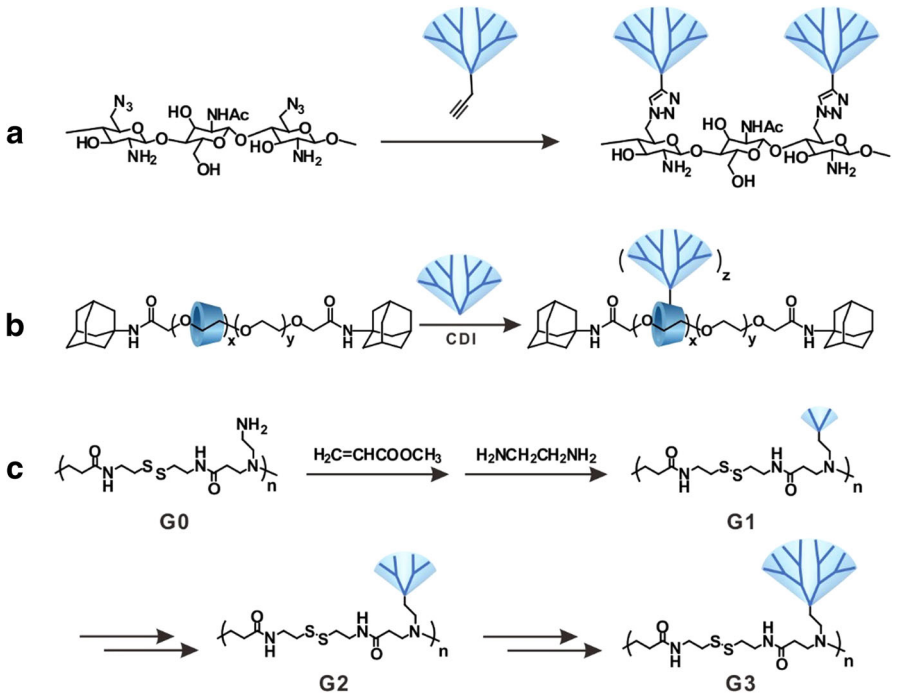

Fig. 8 Grafting low molecular weight dendrons or dendrimers onto the backbone of chitosan (**a**) or polyrotaxane (**b**). Grafting low molecular weight dendrons from a bioreducible polymer to generate a dendronized polymer (**c**)

backbone of a chitosan via click chemistry (Fig. 8a). The dendronized chitosan showed enhanced transfection efficacy compared to free chitosan, low molecular weight dendrons, and polyethyleneimine (PEI) [73]. A G1 PAMAM-grafted polyrotaxane showed higher transfection efficacy, but less cytotoxicity than PEI on different cell lines (Fig. 8b) [75]. The dendrimer-grafted polyrotaxane formed stable polyplexes around 100 nm with plasmid DNA, and the polyplexes were internalized into transfected cells via a caveolae-dependent pathway, which avoided lysosomal degradation after cellular uptake. In addition, the polyplexes were very stable and could maintain the nanoparticle size after 256-fold dilution. This dilution-stable property is essential for in vivo gene therapy because the polyplex solution will be highly diluted when administrated by intravenous injection [75].

Besides the "grafting onto" strategy, we can also adopt a "grafting from" method to fabricate dendrimer-anchored polymers [76–78]. Low-generation dendrons could be synthesized on the scaffold of a linear polymer scaffold by a stepwise manner. By synthesizing low-generation PAMAM dendrons on a disulfide-containing polymer via repetitive Michael addition and amidation, a series of bioreducible dendronized polymers were obtained (Fig. 8c) [76]. Since the high charge density on the dendronzied polymer, the material showed strong DNA binding ability and greatly improved transfection efficacy compared to low molecular weight dendrimers. In addition, the dendronized polymer with a reduction-sensitive polymer scaffold was able to degrade into low molecular weight species in the presence of thiols or acids, which allows limited toxicity on the cells. Similarly, peptide-based dendrons were synthesized on a bioreducible polymer to obtain a dendronized polymer for siRNA delivery. The generated polymers also exhibited high delivery efficiency in serum and low toxicity on the cells, and would be a promising non-viral carrier for siRNA delivery [77]. These strategies encourage further design of hybrid materials consisted of low molecular weight dendrimers or dendrons and biocompatible nanoparticles, proteins, or polymers for efficient gene delivery.

4 Crosslinking Low-Generation Dendrimers into Nanoaggregates or Polymers

Low molecular weight dendrimers could be cross-linked into larger nanoclusters by covalent linkages for improved gene transfection. For example, the surface of a G2 PAMAM dendrimer was engineered with multiple G1 PAMAM dendrimers via polyglutamate linkers [79]. The material constructed by G1, G2 dendrimer and polyglutamate showed high efficacy and excellent serum-resistance in gene delivery. In a separate study, G2 PPI dendrimers were reacted with 10-bromodecanoic acid to increase the lipophilicity, and the carboxyl groups on the terminal of aliphatic chain were further reacted with residual amine groups on G2 PPI dendrimer, yielding a nanocluster with improved transfection efficacy [80]. However, low molecular weight dendrimers were crosslinked via non-degradable linkages in these nanoclusters. Though the transfection efficacy is highly improved,

the cytotoxicity is still a problem for the nanoclusters due to high charge density on the materials.

If the low molecular weight dendrimers were crosslinked using a stimuli-responsive linker, the fabricated nanoclusters could be degraded into small dendrimers with minimal toxicity in acidic or reductive microenvironments after cell endocytosis. For example, G2 PAMAM dendrimer cross-linked by a disulfide containing linker formed nanoclusters around 40 nm (Fig. 9a). The disulfide-crosslinked G2 dendrimer showed much improved transfection efficacy compared to intact G2 dendrimer, and was superior to a G5 dendrimer [21]. An analogue material cross-linked by a non-degradable linker showed extremely low transfection efficacy, suggesting that the reduction-sensitive property of the degradable linker is essential for efficient gene delivery. Surprisingly, the disulfide-crosslinked G2 dendrimer was even less toxic than intact G2 dendrimer on the transfected cells, probably due to the high density of cationic charges in the interior of fabricated nanoclusters. The cationic charges located in the interior will not cause damage to the cells, and thus minimal toxicity was observed for the synthesized materials [21]. Similarly, disulfide-crosslinked G1 polylysine dendrimer showed high transfection efficacy and low cytotoxicity on different cells [81]. By the combination of fluorination and disulfide crosslinking, an efficient nanocluster was prepared for in vitro and in vivo gene delivery with several unique features, e.g.. inactive surface to resist protein interactions due to the presence of fluorous chains; virus-mimicking surface

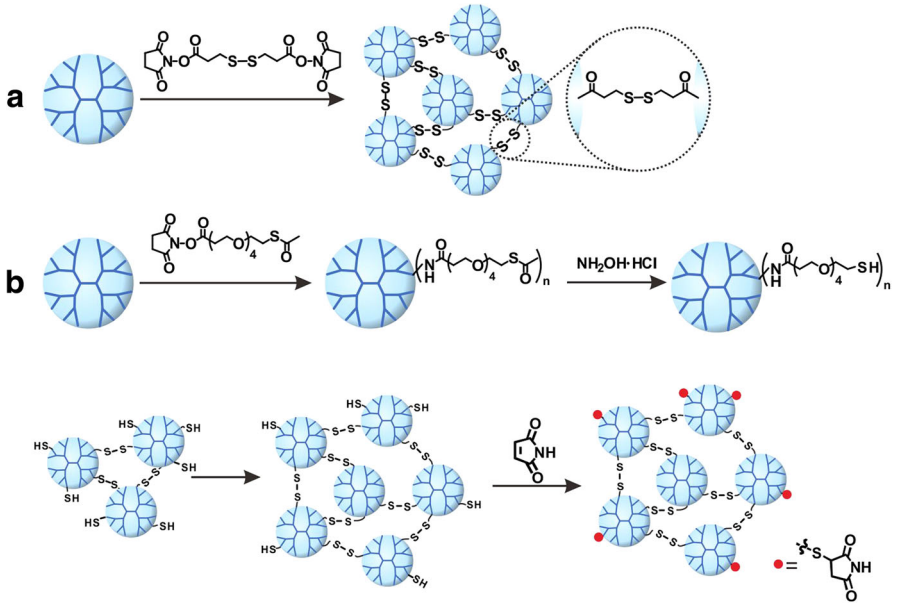

Fig. 9 Crosslinking a G2 PAMAM dendrimer into a bioreducible nanocluster for efficient gene delivery (**a**). Reproduced from Ref. [21] with permission from ACS 2012. A strategy used to control the size of disulfide crosslinked nanoclusters (**b**). Initiating the crosslinking reaction by deprotecting the thiol groups on dendrimer surface, while stopping the crosslinking reactions by eliminating the remaining thiols through the addition of excess maleimide. Reproduced from Ref. [83] with permission from ACS 2012

topography to augment cellular uptake; a fluorous effect mediated efficient cellular uptake, endosome escape, and cytoplasm trafficking; and glutathione-triggered nanocluster degradation and intracellular DNA release [82]. These features together contributed to high efficacy of the fabricated nanoclusters. The studies concluded that disulfide-crosslinked low-generation dendrimers with high transfection efficacy, low toxicity, and low cost are efficient alternatives to high generation dendrimers in gene delivery.

One remaining problem for the disulfide-crosslinked nanoclusters is the difficulty in controlling the nanocluster size. The final nanocluster size depends on lots of parameters such as the ratio of linker to dendrimer, the addition rate of linkers, solvents, and reaction temperature, etc. Therefore, the reproducibility of nanoclusters by this strategy is still a sticky issue. We can address this issue by adopting an alternative strategy to prepare disulfide-crosslinked dendrimer. Low molecular weight dendrimers were firstly modified with protected thiols on its surface, and then the crosslinking reactions were initiated by deprotecting the thiol groups in aqueous solution. The nanocluster size was monitored by dynamic light scattering and the cross-linking reactions could be stopped by eliminating the remaining thiols on dendrimer surface through the addition of excess maleimide molecules (Fig. 9b) [83]. By this strategy, disulfide-crosslinked nanocluster with a desirable size in the range of tens to hundreds of nanometers could be obtained.

Besides using stimuli-responsive linkers, nanoclusters were fabricated by mixing two kinds of hyperbranched polymers with different surface functionalities [84, 85]. A low molecular weight hyperbranched oligoethylenimine modified with phenylboronic acid could react with 1,3-diol-rich hyperbranched polyglycerol via boronic acid-diol linkage, forming a dynamic and reversible nanocluster with relatively high charge density (Fig. 10). The nanocluster exhibited strengthened binding affinity to siRNA compared to low molecular weight oligoethylenimine. The degradation of nanocluster could be triggered by lysosomal acidity, which ensures minimal toxicity of polymers on the cells. In addition, the hydrophobic interior of hyperbranched polyglycerol could be loaded with anticancer drugs such as doxorubicin for synergistic cancer therapy. Very recently, the same group adopted a similar strategy to construct acidity-responsive nanoclusters based on specific recognition between phenylboronic acid and diols [86]. Phenylboronic acid-conjugated cholesterol and hyperbranched oligoethylenimine modified with 1,3-diols formed nanoclusters through a combination of hydrophobic interaction between cholesterols and boronic

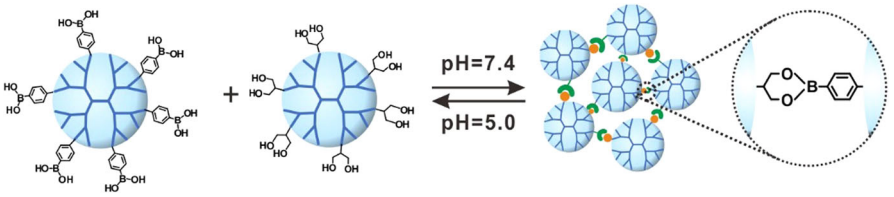

Fig. 10 Fabrication of nanocluster via the covalent and reversible linkages between phenylboronic acid and 1,3-diols on the surface of low molecular weight hyperbranched polymers. Reproduced from Ref. [84] with permission from Elsevier 2015

acid-diol recognition. The formed nanocluster also showed efficient gene transfection and low toxicity in vitro and in vivo.

5 Conclusions

We can fabricate low molecular weight dendrimers or dendrons into different nanostructures for efficient and low toxic gene delivery. The low molecular weight polymers could be assembled into nanoaggregates via ionic, hydrogen-bonding, hydrophobic, or fluorophilic interactions. Alternatively, the low molecular weight polymers could be anchored to biocompatible nanoparticles, proteins, and polymers to generate hybrid nanostructures, or be crosslinked into nanoclusters with stimuli-responsive property. These nanostructures can form stable polyplexes with DNA or siRNA in vitro, and degrade into low molecular weight species in the presence of specific triggers such as endolysosomal acidity, glutathione, and enzymes. The flexible strategies available in the design of supramolecular or hybrid nanostructures allow the design of nanostructures with high transfection efficacy and minimal toxicity on the cells. Though these supramolecular polymers showed impressive efficacy in vitro, further modifications on the assembled polymers or nanostructures are needed to shield the positive charges on the polyplexes to transfer the materials for in vivo gene delivery. For example, we need to attach the polyplex with a responsive polyethylene glycol (PEG) shell to improve its blood circulating time, or with a hyaluronic acid shell to improve its accumulation in breast tumors overexpressing CD44. The technologies to fabricate low generation dendrimers into highly efficient and low toxic nanostructures will have broad applicability for clinical applications.

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