Expert Opinion

Design of stimuli-responsive dendrimers

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Importance of the field: Dendrimers are synthetic macromolecules with well-defined structures, many terminal functional groups, and an inner space to hold small molecules. These properties make them potential drug carriers. Recently, stimuli-responsive drug delivery systems have become attractive because of the reduction of side effects and maximum expression of drug action.
Areas covered in this review: This paper reviews dendrimer nanoparticles that are sensitive to temperature, light, pH and redox state.
What the reader will gain: Strategies to design these dendritic polymers are provided in this review.
Take home message: By adding stimuli-responsive properties to the dendrimers, dendritic polymers capable of controlled release can be produced. These stimuli-responsive dendrimers are a potential next generation drug carrier.

Keywords: dendrimer, photo-sensitive, pH-sensitive, redox-sensitive, temperature-sensitive

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1. Introduction

Drug delivery systems (DDS) are useful for reducing drug side effects and maximizing drug action. The design of drug carriers for DDS is the most important research issue in this area. A simple drug carrier is a nanocapsule containing drug molecules. Even though these nanoparticles could reduce side effects, drugs may not effectively access their site of action. Therefore, the addition of a controlled release system to a drug carrier is useful. Various stimuli-responsive drug carriers have been reported [1-10]. These carriers are composed of moieties sensitive to various stimuli such as temperature, pH, light and redox state. Temperature and light can be applied to areas of the body as external stimuli, and are already clinically used. By contrast, pH and redox state are self-regulated internal body stimuli, and differences in pH and redox state are observed between various tissues and/or subcellular compartments. Drug carriers responsive to these stimuli could be useful for controlled drug release [1-10].

Several different nanoparticles such as liposomes, micelles and polymers have been adopted as drug carriers [1-12]. Liposomes and micelles are self-assembled nanoparticles, whereas polymers can be unimolecular nanoparticles that are more stable than self-assembled nanoparticles. In comparison with liposomes and micelles, polymers are smaller and similar in size to proteins. This should allow polymers to penetrate deeply into tissues. In general, synthetic polymers cover a broad molecular mass range and have uncontrollable conformation, which makes them quite different from biomacromolecules such as proteins. Dendrimers are attractive synthetic macromolecules with monodispersed and unique structures. Recently, these polymer nanoparticles have been studied as a new type of drug carrier [12-22]. This review focuses on the application of dendritic polymers to stimuli-responsive DDS. A brief explanation of dendrimer properties as drug carriers is provided. Various types of stimuli-responsive dendrimer and the design of stimuli-sensitive dendritic polymers are discussed.

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Article highlights.

- An overview of stimuli-responsive nanoparticles for drug delivery systems.
- An overview of dendrimer properties.
- Examples of various temperature-dependent dendrimers and the molecular design.
- Examples of various pH-dependent dendrimers and the molecular design.
- Examples of various redox-dependent dendrimers and the molecular design.
- Examples of various photo-dependent dendrimers and the molecular design.
- Indispensable studies for practical applications of stimuli-responsive dendrimers to drug delivery.

This box summarises key points contained in the article.

2. Dendrimers

2.1 General properties

Dendrimers were first synthesized in 1985 [23]. They have a core, building blocks and terminal groups, and their molecular mass increases with increasing generation number (**Figure 1**). Their properties can be controlled by choosing a suitable core, building blocks or terminal groups. Dendrimers have been the focus of a wide range of research [12-22]. To design stimuli-sensitive dendritic polymers, tunable moieties can be modified at the terminal groups and/or the core. The dendrimer backbone and encapsulated molecules can also act as stimuli sensors.

Many types of dendrimer have already been produced [12-22]. One of the most popular is the polyamidoamine (PAMAM) dendrimer made by Tomalia and co-workers [23,24]. This dendrimer is synthesized by repeating reactions of Michael addition and amidation reactions in a divergent manner, and contains many tertiary amino groups and amide groups. A variety of PAMAM dendrimers have been produced with different core structures and terminal groups. The terminal groups used for the PAMAM dendrimers are amino, carboxyl and hydroxyl groups and the core structures are ethylenediamine, 1,4-diaminobutane, 1,6-diaminohexane, 1,12-diaminododecane, and cystamine. These dendrimers are commercially available from Sigma-Aldrich (St Louis, MO, USA). Other popular dendrimers for DDS include poly(propylene imine) (PPI) dendrimers, which are also called diaminobutane (DAB) dendrimers, polyester dendrimers and poly-L-lysine dendrimers. PPI dendrimers also have tertiary amino groups, similar to PAMAM dendrimers. Polyester and poly-L-lysine dendrimers are biodegradable dendrimers, which is a very useful property for DDS. Poly-L-lysine dendrimers are different from PAMAM, PPI and polyester dendrimers. The former dendrimers have unsymmetrical branched structures, but the latter ones have symmetrical ones. Small generation PPI and poly-L-lysine dendrimers are also commercially available from Sigma-Aldrich, and various types of polyester dendrimer with different terminal groups and core structures can be purchased from Polymer Factory (Nacka, Sweden).

2.2 Usefulness as a drug carrier

Dendrimers are suitable as drug carriers because of their reproducible synthesis, potential to be passively targeted to cancer tissue, and drug loading capability. The controllable and uniform molecular mass, size and chemical composition of dendrimers contribute to their chemical and biological reproducibility. For cancer therapy, the size of a drug carrier is crucial for passive targeting because of the enhanced permeability and retention (EPR) effect, which derives from the permeable blood vessel endothelium and the lack of lymphatics in tumor tissues [25,26]. Size-controlled nanoparticles can be targeted to tumor tissues using the EPR effect. Therefore, dendritic nanoparticles can passively accumulate in tumor tissues owing to EPR effects [12-17]. Dendrimers can include drug molecules through either encapsulation or conjugation [13-20]. Several drug molecules can be solubilized and encapsulated by dendrimers, including drug molecules such as doxorubicin, methotrexate, 5-fluorouracil and paclitaxel [13-20]. Preparation of drug-encapsulating dendrimers is generally performed by simple mixing. Drug molecules are associated with dendrimers by means of hydrophobic interaction, electrostatic interaction, hydrogen bond, and so on [13-20]. Therefore, the dendrimer structures largely affect the affinity with drug molecules. The dendrimer symmetry is also important for drug encapsulation. It was reported that poly-L-lysine dendrimers do not possess interior void space for encapsulation because of the unsymmetrical structure [24,27]. Also, some drug molecules can conjugate with the terminal group of the dendrimer. Owing to encapsulation and conjugation, dendritic nanoparticles can be loaded with drug molecules for application to DDS [13-20].

How can we design stimuli-responsive dendrimers for controlled drug release? In the case of drug-encapsulated dendrimers, drug molecules are associated through hydrophobic interaction, electrostatic interaction and hydrogen bonds. Therefore, stimuli-responsive DDS can be promoted by changes in hydrophobicity, charge and chemical composition of the dendritic polymer. These changes could be induced by stimuli such as temperature, light, pH and redox states. In the case of drug-conjugated dendrimers, the linkage between the drug and dendrimer is very important. Acid-degradable bonds and disulfide bonds are cleaved under acidic and reducing conditions, respectively. Drugs conjugated to dendrimers by means of these degradable linkages are also useful for stimuli-responsive DDS.

3. Temperature-responsive dendritic polymers

Hyperthermia therapy (thermotherapy) involves exposure of cancer cells to high temperatures to damage them. This therapy can be achieved by clinically approved radiofrequency ablation as a local heating system [28]. Temperature-responsive DDS can be applied in conjunction with thermotherapy. There are already many reports on different types of temperature-sensitive nanoparticle. Application of temperature-sensitive polymers, of which poly(*N*-isopropylacrylamide) (PNIPAM)

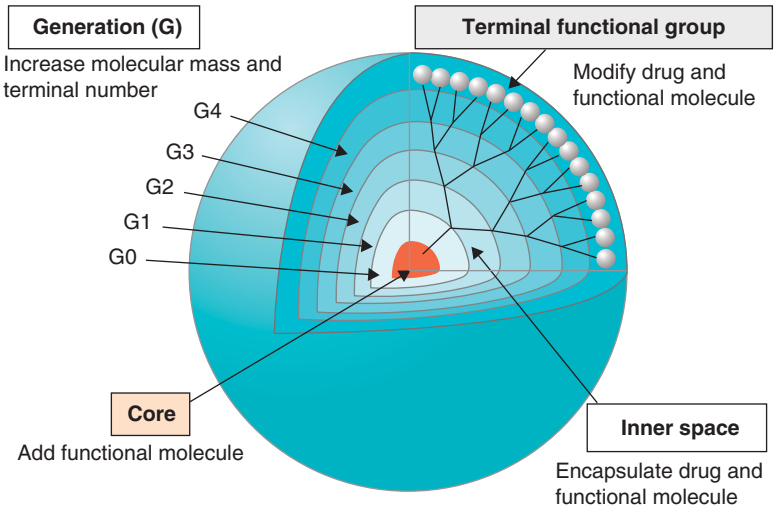

Figure 1. Structure of a dendrimer.

is representative, takes advantage of the design of thermosensitive DDS [1-6]. These polymers have a phase transition at which the solubility drastically decreases. The 'cloud point' or lower critical solution temperature (LCST) of PNIPAM is 32°C [1-6]. Polymers containing an ethylene glycol unit, such as poly(2-(2′-methoxyehoxy)ethyl methacrylate), are also temperature-sensitive polymers. The cloud point is heavily influenced by the balance between hydrophobicity and hydrophilicity of the polymer [1-6]. These thermosensitive polymers are useful for the preparation of temperature-sensitive dendrimers. For example, temperature-sensitive polymers can be attached to the core or the terminal groups of the dendrimer (**Figure 2A, B**). Interestingly, modifying only a part of these polymers, such as isobutyl amide and *N*-isopropylamide within the dendrimer, was enough to impart temperature-sensitive properties (**Figure 2C**). Polymers containing the ethylene oxide unit have also shown temperature-responsive properties (**Figure 2D**).

Collagen is a temperature-sensitive protein composed of glycine-proline-(hydroxy)proline (Gly-Pro-Pro(Hyp)) repeats that form a triple helix [29-31]. At its melting point, collagen becomes gelatinous as a result of dissociation of the triple helix. The temperature-dependent behavior of collagen is different from temperature-sensitive synthetic polymers having a LCST. Therefore, collagen-related materials can provide an alternative temperature-dependent material. It has been reported that a dendrimer modified with collagen model peptides showed temperature-sensitive properties [32]. In this section, various types of temperature-sensitive dendritic polymer are described.

3.1 Dendrimers modified with thermosensitive polymers

As mentioned previously, temperature-sensitive polymers such as PNIPAM are very useful for the design of temperature-dependent dendrimers. Two strategies have been used to modify dendrimers with these polymers. The first involves modification of the polymer at the surface (**Figure 2A**), and the second involves modification at the core (**Figure 2B**).

Kimura *et al.* reported the first temperature-responsive dendrimer, which was prepared by polymerization of NIPAM from the termini of a PPI dendrimer with terminal thiol groups [33]. The core of the dendrimer was a cobalt complex, which catalyzed oxidation of the thiol compounds. They showed that this reaction could be controlled by temperature [33]. You *et al.* reported a dendrimer modified with PNIPAM via reversible addition-fragmentation chain transfer (RAFT) polymerization [34]. As RAFT polymerization is a precise polymerization technique, the molecular mass range of the PNIPAM-modified dendrimer was relatively narrow [34]. These PNIPAM-modified dendrimers cannot be applied as DDS, because the transition temperature of PNIPAM is 32°C. Temperature-dependent dendrimers with higher cloud points of ~ 40°C are necessary. The hydrophobic–hydrophilic balance in thermosensitive polymers is crucial in determining the cloud point. Dendrimers modified with PNIPAM-*b*-poly (dimethylaminoethyl methacrylate (PDMA) were synthesized to adjust the cloud point. The addition of hydrophilic polymers induced responsiveness at higher temperatures [35]. Yang *et al.* reported on both PNIPAM and polyethylene glycol (PEG)-modified dendrimers at different ratios, and the binding ratio of these polymers affected the cloud point of the dendrimer [36]. Recently, they also reported that polycaprolactone-*b*-PNIPAM-modified dendrimer was a temperature-dependent nanocapsule of daidzein, a traditional Chinese medicine. In this dendrimer, polymer layers of polycaprolactone and PNIPAM acted as a drug reservoir and a temperature sensor, respectively [37].

In addition to surface modifications, the temperature-sensitive polymer core also plays an essential role as a temperature sensor for dendritic polymers (**Figure 2B**).

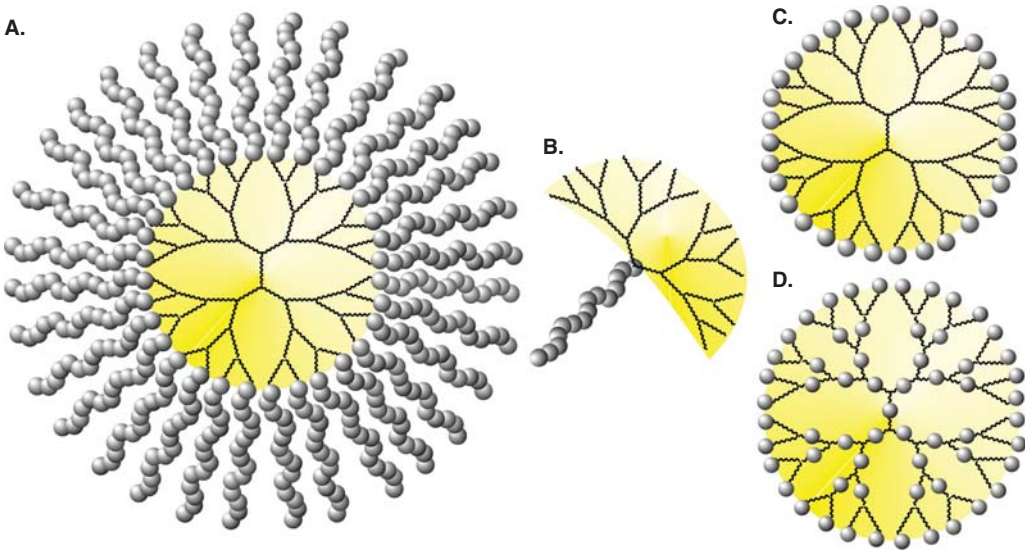

Figure 2. Design of temperature-sensitive dendritic polymers. A. Dendritic polymers attached to a temperature-sensitive polymer at the surface. **B.** Dendritic polymers with a temperature-sensitive polymer core. **C.** Dendrimers modified with a temperature-sensitive moiety. **D.** Dendritic polymers composed of a temperature-sensitive moiety. A single ball and linked balls indicate a temperature-sensitive moiety and a temperature-sensitive polymer, respectively.

From the core of polyol dendrons, NIPAM was polymerized to produce a temperature-dependent dendritic polymer. These polymers underwent temperature-dependent self-assembly [38,39]. Lee *et al.* reported that 2-(2′-methoxyethoxy)ethyl methacrylate was polymerized from a core of a polyester dendron. This block polymer also showed temperature-dependent properties. In addition, PEG was attached to the termini of the temperature-dependent dendrimer to increase the phase transition temperature [40]. Stover *et al.* reported the controlled release of a proapoptotic drug, ceramide, using a temperature-sensitive dendritic polymer [41]. This polymer was made by polymerization of L-lactide and NIPAM from the core of a poly-L-lysine dendrimer [42]. The core block polymer associated with ceramide, and this was dependent on temperature. In addition, the cellular uptake of the dendritic polymer was also sensitive to temperature. This nanoparticle induced cytotoxicity to a similar degree as the free drug and the liposomal drug at 37°C [41].

3.2 Dendrimers containing thermosensitive moieties

As mentioned already, the modification of temperature-sensitive polymers to dendritic polymers imparted temperature-responsiveness to the dendrimer. Interestingly, temperature-sensitive dendrimers could be prepared by modifying only a part of a temperature-sensitive polymer at the surface. Kono's group has studied this kind of dendrimer [43-47]. They first reported on temperature sensitivity for PAMAM and PPI dendrimers by modification with isobutyric acid to provide the isobutylamide (IBAM) group on the dendrimer surface [43]. The temperature sensitivity was largely dependent on the generation or molecular mass. This property was quite different from thermosensitive linear polymers. In addition, these dendrimers were also influenced by pH because of their inner tertiary amine [43]. To compare a typical linear temperature-sensitive polymer, PNIPAM, with these temperature-sensitive dendrimers, dendrimers with *N*-isopropylamide at the surface were synthesized by reacting isopropylamine with carboxylic acid-terminal PAMAM dendrimers [44]. Linear PNIPAM has an endothermal peak near the cloud point, which is attributed to dehydration from the polymer. By contrast, these dendrimers had an extremely small endothermal peak. Therefore, this kind of temperature-dependent dendrimer is very unique and different from the linear structured temperature-sensitive polymers [44]. PAMAM dendrimers were also modified with phenylalanine (Phe) instead of *N*-isopropylamine and IBAM [45]. This dendrimer also induced thermosensitivity under physiological pH, but dendrimers with attached leucine and isoleucine did not. Tuning of the phase transition temperature to approximately body temperature is crucial for application of these dendrimers to DDS. In Phe-bound dendrimers, the bound ratios of Phe to the dendrimer contributed to the cloud point [45]. Dendrimers containing hydrophobic amide groups such as cyclopropanecarboxylic acid amide (CPCAM) had lower cloud points. The dendrimers at an IBAM/CPCAM bound ratio of 1:1 also had a cloud point around body temperature [46]. One of advantages of dendrimers is their ability to encapsulate small molecules. The influence of a guest molecule, rose bengal, on the temperature sensitivity was also investigated [47]. A greater number of guest molecules loaded in the dendrimer decreased the cloud point. This suggests that guest molecules enhanced the hydrophobicity of the dendrimer [47]. Therefore, the guest

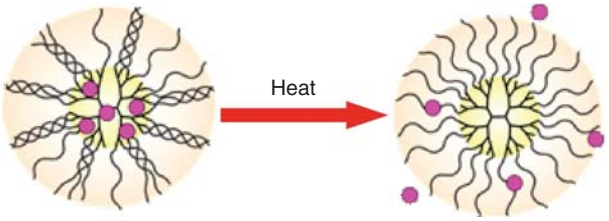

Figure 3. Collagen-mimic dendrimers at different temperature. Derived from [32].

molecule can also control the thermosensitivity of temperature-sensitive dendrimers.

Asthmanikandan *et al.* [48] and Chang and Dai [49] reported oligo(ethylene glycol)-bound dendritic compounds that had temperature-dependent phase transitions, such as the amide group-containing dendrimers. These dendrimers were composed of hydrophobic and hydrophilic parts to form a dendritic micelle, whose morphology responded to temperature. Asthmanikandan *et al.* reported that Rhodamine 6G could be encapsulated by these dendritic micelles [48]. Oligo (ethylene glycol)-bound dendritic polymers are another class of candidate materials for temperature-sensitive DDS.

Dendrimers with a properly balanced hydrophobic backbone can also act as temperature-sensitive dendrimers. Li *et al.* reported on oligo(ethylene glycol)-containing dendrimers as temperature-responsive dendrimers [50]. The thermosensitivity was dependent on both the generation and the terminal structure. The cloud points of the methoxy-terminated dendrimers were higher than those of the ethoxy-terminated dendrimers. In the case of conventional linear and dendritic temperature-sensitive polymers, larger polymers had a lower cloud point because the hydrophobicity in larger polymers was enhanced. In Li *et al.* 's dendrimer, by contrast, the larger generation dendrimers with higher molecular masses had a higher cloud point [50]. Therefore, this kind of dendrimer is a very unique temperature-sensitive polymer.

Parrott *et al.* reported that a polyester dendrimer containing a boron cluster ($B_{10}H_{10}$) showed thermosensitive properties, which were dependent on the generation [51]. As the boron cluster is useful for boron neutron capture therapy (BNCT) [14,15], this dendrimer can participate in both temperature-sensitive DDS and BNCT.

3.3 Collagen-mimic dendrimers

Collagen is the most abundant protein in mammals and a classical biomaterial [29-31]. It is a temperature-sensitive material, which makes it useful for temperature-sensitive DDS. Collagen forms a triple helix to function under physiological conditions. It is difficult for short collagen peptides to form a triple helix, which limits development of artificial collagen material. However, a covalent knot of collagen peptides has been reported to induce triple helix formation [29]. Kinberger *et al.* used a dendrimer as a knot where the terminal groups were modified with a collagen model peptide, (Gly-Pro-Nleu (*N*-isobutylglycine)). These dendrimers induced a collagen-like triple helix formation [52,53]. Recently, the author has also reported a collagen model peptide ((Pro-Pro-Gly)$_5$)-attached dendrimer [32]. The peptides knotted at the surface of the dendrimer also formed a collagen-like triple helix. Interestingly, unlike in natural collagens, the helix formation with this dendrimer was thermally reversible. The collagen-mimic dendrimer could encapsulate a model drug, rose bengal (RB), and the release of RB from the collagen-mimic dendrimer was enhanced at high temperature. This thermosensitivity was based on a temperature-dependent helix formation, not on a phase transition. The collagen-like triple helix formation at lower temperature may improve the binding properties of RB to the dendrimer and/or suppress the permeability of RB at the surface (**Figure 3**) [32].

4. pH-Responsive dendritic polymers

Although physiological pH is 7.4, the pH in the human body varies and can be utilized for pH-responsive DDS. When considering oral DDS, there is a pH difference between the stomach (about pH 2) and intestine (pH 5 – 8). Cancer and inflammation tissues are slightly acidic at pH 6.5 – 7.2, whereas intracellular cytosol, endosome and lysosome pH values are 7.4, 5.0 – 6.5 and 4.5 – 5.0, respectively [6].

There are many types of pH-sensitive nanoparticle that incorporate pH-tunable moieties. These moieties can include carboxyl and/or tertiary amino groups, which function as pH sensors because their hydrophobicity is altered by protonation and deprotonation [1-4,6,10,15,19]. An influenza virus is a good example of a pH-sensitive bionanoparticle; it contains a pH-sensing protein called hemagglutinin (HA). Conformational change of HA is induced by the protonation of carboxyl groups at low pH, and this allows the virus to avoid endosomal degradation [54]. These examples illustrate how dendrimers including pH-sensing moieties on their surface, in their core or in their inner building blocks would be useful for pH-responsive DDS.

pH-Sensitive linkages such as hydrazide and acetal are also useful for pH-sensing systems. Dendrimers conjugated to drugs by means of a pH-sensitive linkage have been studied [10,12,13,15]. The change in hydrophobicity after the cleavage of the pH-sensitive bonds can induce conformational change of the dendritic polymers [10]. A dendrimer has been synthesized from building blocks composed of pH-degraded linkages [55]. In this section, various types of pH-sensitive dendritic polymer are discussed for the design of pH-responsive DDS.

4.1 Dendritic polymers containing pH-responsive moieties

Pistolis *et al.* published the first report on pH-responsive dendrimers in 1999 [56]. They studied the encapsulation of pyrene in PPI dendrimers at different pH. PPI dendrimers have many tertiary amines that are protonated at low pH,

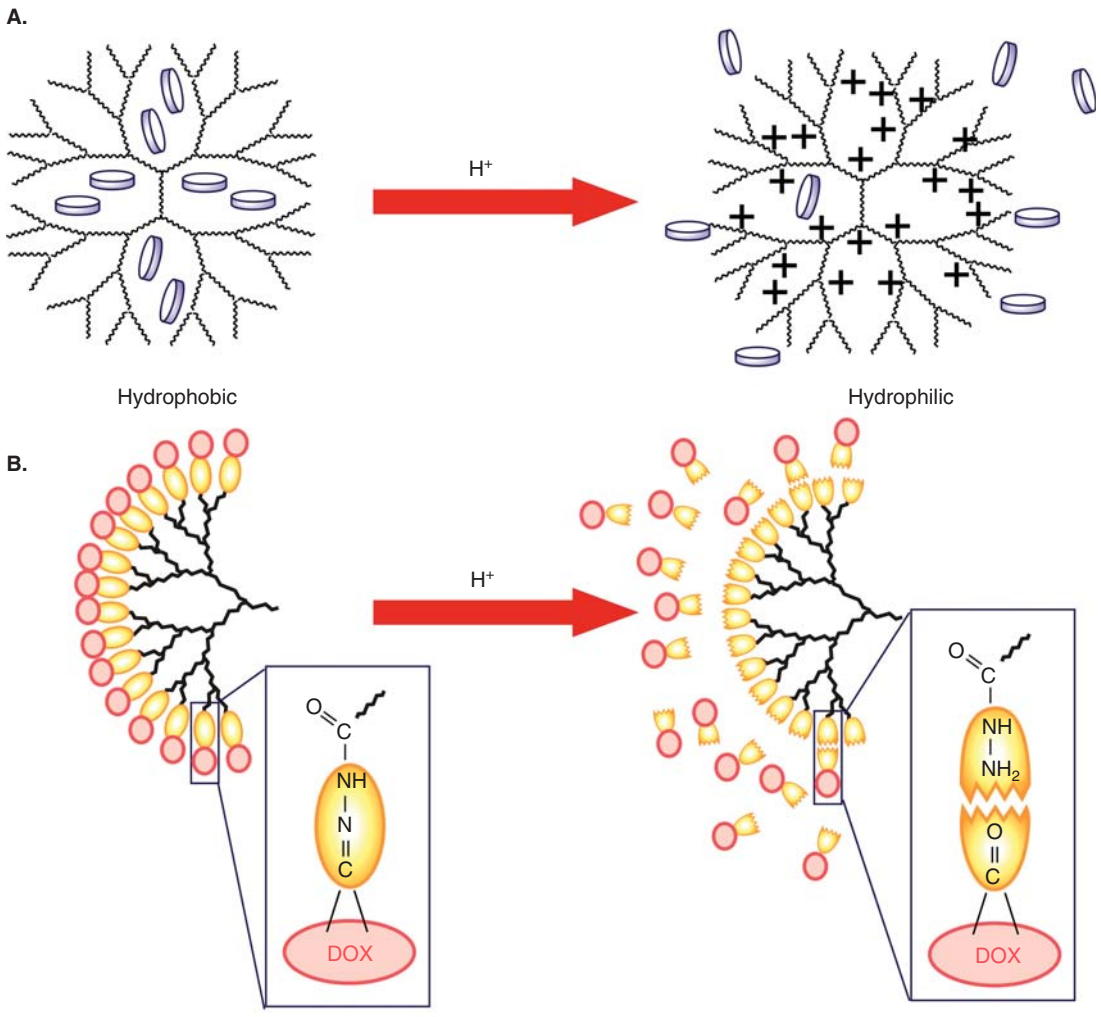

Figure 4. Two major types of pH-responsive dendrimer. A. Drug-encapsulated pH-sensitive dendrimers. Drug molecules can be released from these dendrimers at low pH owing to the reduced hydrophobicity of the dendrimer. **B.** Drug-conjugated dendrimers via pH-dependent hydrazone linkages. This linkage can be cleaved to release the drug molecule at low pH. Dox is doxorubicin.

which decreases the dendrimers' inner hydrophobicity. This facilitated release of hydrophobic pyrene molecules from these dendrimers at low pH (**Figure 4A**) [56]. They also reported on PPI dendrimers with various terminal groups such as quaternized amino groups and PEG chains [57-59]. Gajbhiye *et al.* reported that PEGylated PPI dendrimer could encapsulate an anti-inflammatory drug, aceclofenac, and release it in a pH-dependent manner [60]. Recently, they also reported that PEGylated PPI dendrimer could rapidly release encapsulated famotidine, a model H_2 receptor antagonist, at low pH [61]. PAMAM dendrimers could also be used as pH-sensitive dendrimers because they contain tertiary amines. They were found to solubilize 2-naphthol, nifedipine and nicotinic acid in a pH-dependent manner, with higher drug solubility at high pH than at low pH [62-64]. Tekade *et al.* reported that PEGylated PAMAM dendrimer could encapsulate methotrexate and all-*trans* retinoic acid (ATRA), releasing it from the dendrimer in a pH-dependent manner [65]. Kannaiyan and Imae reported that PPI-core PAMAM-shell dendrimers could encapsulate pyrene molecules in a pH-dependent manner [66]. Also, fluorinated PAMAM dendrimers were found to self-assemble in a pH-dependent manner and encapsulate a guest molecule, rhodamine B, more stably at neutral pH than acidic pH [67]. These examples illustrate some pH-sensitive dendrimer candidates.

Dendrimers with attached pH-sensitive polymers were reported by Yuan *et al.* in 2007 [68]. L-Lactide and 2-(*N*,*N*-dimethylamino)ethyl methacrylate polymerized at the terminal group of a dendrimer formed an aggregate in water, whose size was dependent in pH. Chlorambucil, an anticancer drug, was released faster from these dendrimer aggregates at low pH than at high pH [68].

Some linear polymer-dendrimer conjugates have shown pH-sensitive drug release. These polymers were composed of dendrons containing carboxylic acid or acetal linkages, and they formed micelles in water. As carboxylic acid is protonated at low pH and becomes hydrophobic, under acidic conditions these micelle structures changed [69]. Gillies and co-workers used an acetal linkage to join a hydrophobic trimethoxyphenyl group to a polyester dendron with a hydrophilic PEG core. These dendritic polymers formed micelles with a hydrophobic dendron core and a hydrophilic PEG shell [70,71]. A model drug, Nile Red, and an anticancer drug, doxorubicin, were stably encapsulated in the dendritic micelles at pH 7.4. By contrast, at low pH the micelles were degraded because the hydrophobic phenyl groups were separated from the dendron by cleavage of the acetal group. This resulted in release of Nile Red and doxorubicin under acidic conditions [70,71].

Kohman and Zimmerman reported on a new type of dendrimer that also acted in a pH-sensitive manner [55]. This dendrimer was composed of 1,3,5-triazaadamantane (TAA) at each branch point of the dendrimer. It was degraded by addition of HCl, which caused hydrolysis of TAA under the acidic conditions [55].

4.2 Drug-dendrimer conjugates with pH-responsive linkages

As hydrazone linkages are cleaved at low pH, drugs conjugated to dendrimers via this type of linkage are useful for pH-dependent DDS (**Figure 4B**). The author prepared PEG-modified dendrimers and attached doxorubicin by means of either a pH-insensitive amide bond or a pH-sensitive hydrazone bond. When the cytotoxicity of these doxorubicin-conjugated dendrimers was compared, the hydrazone-linked drug-dendrimer conjugate had higher cytotoxicity than that with an amide link [72]. It is suggested that the drug release from the hydrazone-linked conjugate occurred in acidic endosome and/or lysosome. This illustrates how important release of drug molecules from a dendrimer is for efficient drug action.

Polyester dendrimers incorporating PEG-attached dendrons and dendrons conjugated to doxorubicin by means of hydrazone linkages have been reported [73-75]. *In vitro* and *in vivo* experiments with these dendrimers have shown their biodistribution and the resulting drug action [74,75]. These dendrimers were found to accumulate in tumor tissue and efficiently inhibit tumor growth with a single dose. The drug efficiency was similar to a commercially available PEGylated liposome containing doxorubicin [75]. Recently, an improved and simple synthetic method has been developed for a dendrimer incorporating both PEG and doxorubicin [76]. Such a dendrimer would be very promising for DDS.

5. Redox-responsive dendritic polymers

Glutathione is present inside and outside cells at several millimolar and several micromolar concentrations, respectively [8,9]. This indicates that the intracellular environment is much more reducing than the extracellular environment. This difference between oxidative and reductive environments can be used as a stimulus for controlled release. In an oxidative environment, thiol groups form disulfide bonds, which are then broken in a reductive environment. This suggests that thiol-containing molecules will be useful for the design of redox-responsive dendrimers.

Navath and co-workers reported *N*-acetylcysteine-dendrimer conjugates with disulfide linkages [77,78]. *N*-acetylcysteine is prescribed for neuroinflammation; however, it reacts with cysteine residues of natural proteins resulting in extremely low bioavailability. The disulfide-linked dendrimer-drug conjugates had much better antioxidant effects than the drug alone [77,78]. The author synthesized a PEG-attached dendrimer containing cysteine. The disulfide network at the surface of the dendrimer could be degraded under reductive conditions to enhance the drug release (**Figure 5**). The permeability of the disulfide network in the dendrimer to a model drug, RB, was enhanced in a dithiothreitol solution, which mimics the intracellular environment [79].

6. Photoresponsive dendritic polymers

Photoirradiation is another stimulus that can induce cytotoxicity in affected cells. Photo-related therapy includes photodynamic therapy (PDT) and photothermal therapy (PTT). PDT is a new clinical treatment for superficial tumors and age-related muscular degeneration, and was approved in the 1990s. This technique involves the systemic administration of a photosensitive drug and light irradiation to the affected tissue. This photosensitizer generates singlet oxygen after light irradiation, and causes oxidative damage to cells. PDT affects only the irradiated areas because singlet oxygen is short-lived, making it a site-specific and non-invasive treatment [14,15,80,81]. Dendrimer nanoparticles encapsulating or conjugated to photosensitizers have been reported by several groups. Kataoka's group reported that dendritic polymer micelles composed of a photosensitizer-core dendrimer and PEG-block polymers had an efficient PDT effect [81,82]. In their system, the photosensitizer was isolated in the core of the dendrimer so that the photoresponsiveness was not suppressed by aggregation. In addition, the PEG shell played an important role in enhancing biological efficiency, such as the biodistribution. They demonstrated the *in vitro* and *in vivo* effects of these dendritic micelles. They published an excellent review on polymeric micelles of dendrimers incorporating photosensitizers [81]. The author reported PEGylated PAMAM and PPI dendrimers encapsulating photosensitizers such as protoporphyrin IX and RB. The PEGylated PPI dendrimer was stably complexed with protoporphyrin IX because these molecules are more hydrophobic than the PEGylated PAMAM dendrimers and RB. A PEGylated PPI dendrimer encapsulating protoporphyrin IX had the same cytotoxicity as free protoporphyrin IX because of the generation of greater

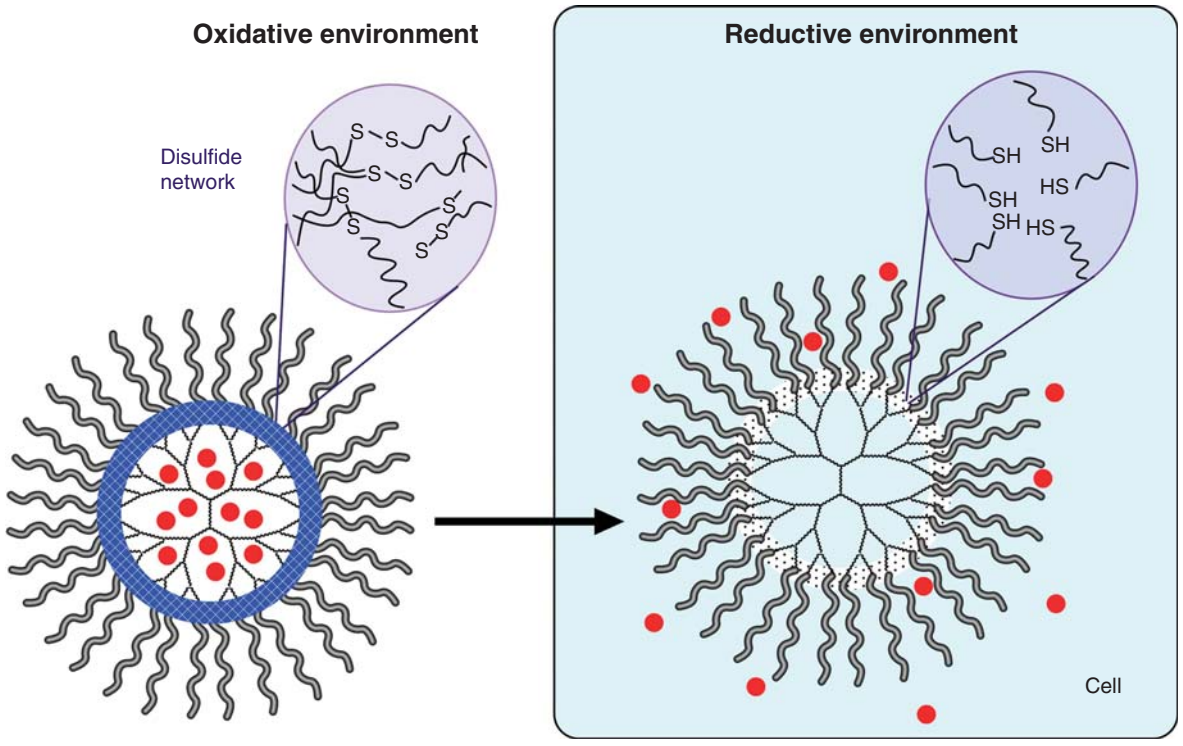

Figure 5. Dendrimers capable of redox-dependent drug release. Derived from [79].

amounts of singlet oxygen and accumulation of the photosensitizer at mitochondria [83]. Battah and co-workers conjugated dendrimers to a precursor of protoporphyrin IX, 5-aminolevulic acid (5-Ala), via an ester bond. 5-Ala was released into cells from the dendrimer by hydrolysis, where it could then form protoporphyrin IX. Cells treated with this dendrimer showed efficient cytotoxicity after light irradiation [84-86]. Oar *et al*. reported on a two-photon system for the excitation of a photosensitizer-conjugated dendrimer. This system is useful for treatment of deeper tissues because of the longer wavelength of the laser [87].

Lai *et al*. reported on photosensitizer addition to hydrazone-linked drug-dendrimer conjugates. They found that drug molecules conjugated to these dendrimers were incorporated into the cytosol in a light-inducible manner. This occurred because the light-activated photosensitizer damaged the endosomal membrane, which allowed endosomal escape of drug [88]. This is called a photochemical internalization [81,88] and this type of dendrimer is a new photosensitive drug carrier.

PTT involves the systemic administration of gold nanoparticles and light irradiation of the affected tissues, similar to PDT with photosensitizers. Gold nanoparticles generate heat after light irradiation, and this damages the cells [14,89-91]. Some biocompatible gold nanoparticles have been studied for PTT [90,91]. It has been reported that a PEGylated dendrimer encapsulating a gold nanoparticle generated photothermal energy, which could be used for PTT [92].

Photo-degradable dendrimers are also useful for photosensitive DDS. Amir *et al*. reported on photosensitive self-immolative dendrimers composed of a photolabile trigger core, model drug compounds at the termini and self-immolative building blocks. The core molecule was activated by photoirradiation to degrade the building blocks of the dendrimer, and the drug molecules were released [93]. Oxidation, reduction and enzymatic reactions can be used as triggers for self-immolative dendrimers; Shabat has reviewed these [94].

7. Conclusion

Dendrimers are unique and well-defined materials. As their molecular mass and chemical composition are theoretically definite, the characterization of newly synthesized compounds is relatively easy. It is worthwhile to develop functional nanoparticles based on dendritic polymers. Dendrimers are particularly useful for systematic synthesis and investigations of functional macromolecules. The design of functional dendritic nanoparticles is important for the next generation of DDS, which is stimuli-responsive DDS. This review summarizes the various functional dendritic polymers that can be produced by addition of temperature-, pH-, photo- and redox-sensitive moieties. Improvement of functional dendritic polymers is required to make them more effective *in vivo*. Dendrimer research has now been active for 24 years since their original synthesis in 1985. It is expected that in the future dendrimers will expand to include potential new drug carriers.

8. Expert opinion

This review discusses many reports of stimuli-responsive dendritic polymers. Most of these involved the preparation of stimuli-responsive dendrimers and the demonstration of controllable release of drugs or model drug molecules. There are some *in vitro* evaluations of functional dendritic compounds using cultured cells. Although *in vivo* studies are indispensable for development of DDS, reports on *in vivo* studies using stimuli-sensitive dendritic polymers have focused on only three areas. The first of these involved a series of polyester dendrimers incorporating doxorubicin via pH-sensitive linkages, and these were studied by Frechet's and Szoka's groups [73-75]; the second involved a series of dendritic micelles for PDT studied by Kataoka's group [81,82]; and recently Gajbhiye *et al*. reported on *in vivo* effects of a PEGylated PPI dendrimer encapsulating anti-inflammatory agents, which is the third study [60]. The dendritic polymers in these areas of study have a common property. They contain the PEG moiety in their nanoparticles. It has been reported that drug carrier modification with PEG could suppress nonspecific cytotoxicity and enable the stealth effect [95]. For cancer therapy, nanoparticles with long blood circulation times can accumulate in tumor tissues by EPR effects [95]. PEGylation of the dendrimer can enhance both blood circulation and accumulation in the tumor tissues [15,17,96]. Therefore, PEGylation of stimuli-responsive dendrimers is crucial for *in vivo* use. The importance of PEGylation of dendrimers has been highlighted in another review [17]. The attachment of ligands such as folic acid, sugar moieties and peptides to dendrimers could improve the accumulation of dendritic nanoparticles at cancer tissues [13-15,18,97]. Kataoka's dendritic nanoparticles are classified as polymeric micelle-based DDS, even though the micelles were composed of photosensitizer-core dendrimers [81,82]. The stimuli-responsive dendrimer with the greatest potential for clinical application is, at present, the polyester dendrimer conjugated to doxorubicin by means of a hydrazone linkage [75]. This dendrimer contained drug molecules and inhibited tumor growth to a similar degree as a clinically approved liposomal DDS material.

Dendrimers can be unimolecular nanoparticles with a similar size to proteins, even though conventional nanoparticles such as liposomes and micelles are self-assembled nanoparticles. Therefore, dendrimer compounds can be beneficial for simple treatment and deep penetration into the tissues. Unfortunately, dendrimer application seems to be expensive. Hyperbranched polymers are a useful alternative to dendrimers because they are simple to synthesize and have a similar globular structure to dendrimers. Temperature-sensitive and pH-sensitive hyperbranched polymers have been reported by several groups [12,98-102]. In addition, extremely low toxicity of the dendritic materials is necessary for DDS application. In this regard, biodegradable dendrimers such as polyester and poly-L-lysine dendrimers and hyperbranched polyglycerol may be useful [13,16,98].

Dendritic polymers have also been incorporated into imaging systems, such as optical imaging and magnetic resonance imaging [103]. When combining stimuli-responsive dendritic polymers with imaging techniques, new functional dendritic polymers are required. Some stimuli-responsive dendritic polymers could be used as potential imaging probes to internal self-regulated stimuli such as pH. Some pH-responsive dendrimers have been applied as pH-imaging probes [67,104]. For DDS responsive to external stimuli, such as temperature and light, the timing of the stimuli application to the affected tissues should largely affect the drug action. Therefore, the development of stimuli-responsive dendritic polymers containing imaging probes is desirable.

Most studies for the application of dendrimers to DDS have been basic and conceptual. It is hoped that future studies will include practical experiments for biomedical applications.

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Declaration of interest

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