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Mini-review

Functional dendritic polymer architectures as stimuli-responsive nanocarriers

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ABSTRACT

Stimuli-responsive polymer architectures are molecular systems which evolve with an external signal. The observed changes are mainly decomposition, isomerization, polymerization, activation, supramolecular aggregation, and structural modifications of these molecules. The external stimuli, which can be combined in order to provoke these molecular changes, are numerous. In this review, we have chosen to present an overview on different mechanisms to impart responsiveness to dendritic polymers, with the particular aim of delivery and release of bioactive molecules.

1. Introduction

The diverse pathophysiological conditions of diseases continually call for innovative and novel therapeutic approaches. In addition to the further development of conventional treatment strategies, the application of nanotechnology in medicine and pharmaceuticals is a rapidly moving field that is gaining fast acceptance and recognition as an independent area of research and scientific endeavor [1,2]. This approach is based on the use of materials which have at least one dimension in the scale range of 1–100 nm for a specific diagnostic or therapeutic purpose. The development of molecular nanostructures with well-defined particle shapes and sizes is of eminent interest in such biomedical applications as the delivery of active pharmaceuticals or imaging agents. Because of their utilization as carriers in drug delivery they should generally be in the nanometer range and uniform in size to enhance their ability to cross cell membranes and reduce the risk of undesired clearance from the body through the liver or spleen. As a result, most of the literature on nanocarrier systems describes the use of polymeric material for therapeutic and diagnostic applications [3,4]. The polymers chosen for the preparation of drug-polymer carriers should ideally be water-soluble, nontoxic, and non-immunogenic, as well as degraded and/or eliminated from the organism [5]. In addition, the polymeric carrier should exhibit suitable functional groups for physical interaction or chemical attachment of the respective drug or spacer fragment. A broad spectrum of synthetic polymers with structural and architectural variations, including linear, starlike, dendritic, and hydrogels is currently under investigation.

An innovative route to create very well-defined, monodisperse, stable molecular level nanostructures is being studied using dendritic polymer architectures [6]. These structures are undoubtedly one of the most pervasive topologies observed throughout biological systems on virtually all dimensional length scales [7]. A comparison of dendrimer and linear polymer features shows that dendritic polymer architecture is advantageous for delivery applications. For example, the controlled multivalency of dendrimers can be used to encapsulate or conjugate similar or different drug molecules while adding-on targeting and/or solubilizing modalites on the same construct in a maneuverable pattern. In addition, their low polydispersity should provide a more reproducible pharmacokinetic behavior than in linear polymers. Furthermore, the more globular shape of dendrimers, as opposed to the random coil structure of most linear polymers, could affect their biological properties and thus lead to the discovery of interesting effects due to their macromolecular architecture [8].

Commercially available polyamidoamine (PAMAM) dendrimers (Fig. 1a), prepared by the divergent growth approach of Tomalia et al. [9], are some of the most widely used dendrimer scaffolds in biology [10]. Despite their broad applicability, it is generally necessary to modify the surface amine groups of these dendrimers with neutral or anionic moieties to avoid the toxicity and liver accumulation associated with their polycationic surfaces [11]. Polypropyleneimine dendrimers (PPI, Fig. 1b) have been

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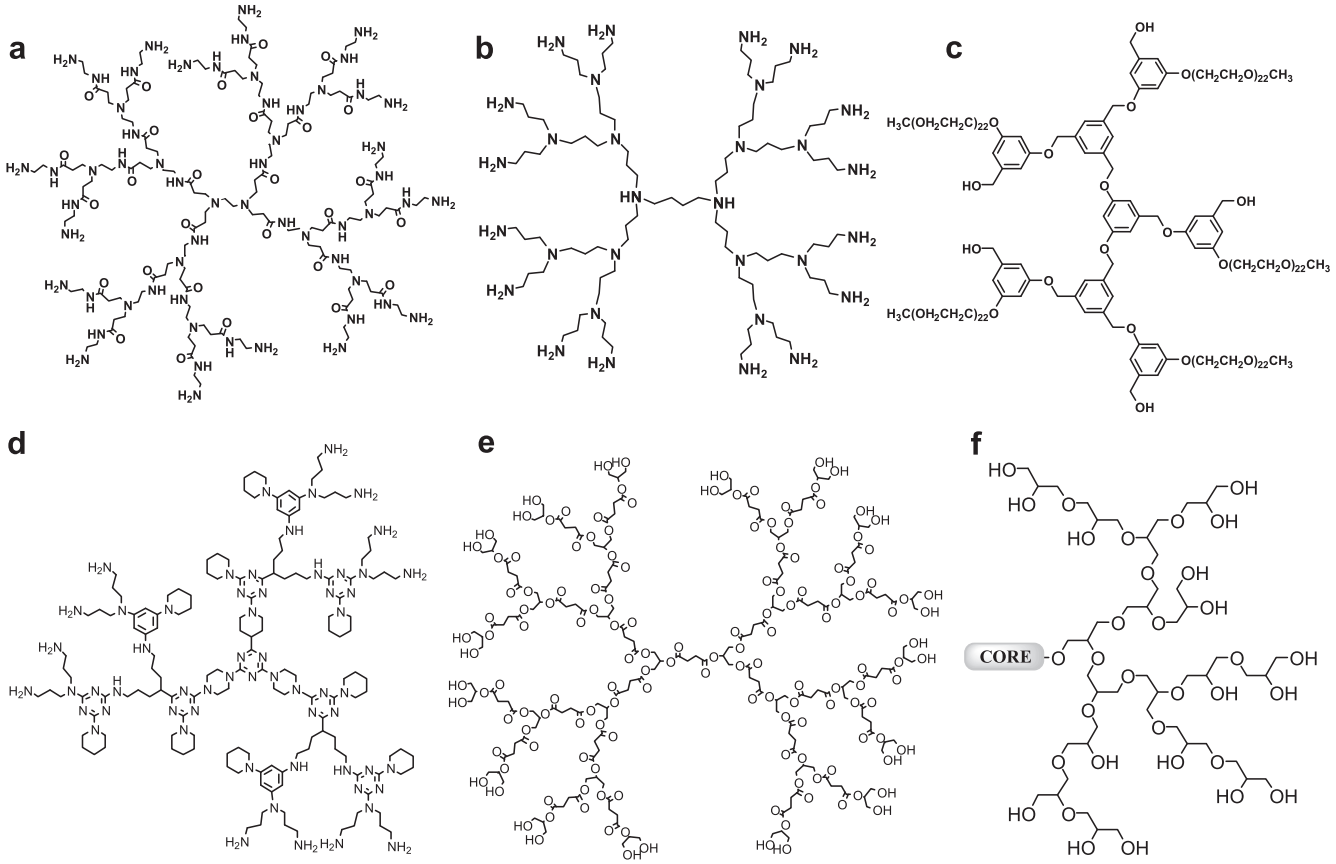


Fig. 1. Examples of dendritic scaffolds commonly used in drug delivery applications. (a) PAMAM, (b) poly(propylene imine), (c) polyaryl ether, (d) triazine-based dendrimer, (e) poly (glycerol–succinic acid) dendrimer, and (f) hyperbranched polyglycerol.

commercialized and investigated for their biological applications, particularly in the field of gene delivery due to the presence of multiple cationic amine groups in their chemical structure [12]. Polyaryl ether dendrimers (Fig. 1c), developed by Fréchet and Hawker [13], have been tested for drug delivery applications although their poor water solubility necessitates the extensive use of solubilizing groups at their periphery [14,15]. Simanek and co-workers have shown the versatility of triazine-based dendrimers (Fig. 1d) for their use in several applications ranging from DNA and RNA delivery to the development of dendritic scaffolds against infectious diseases and cancer [16].

More recently, some dendrimers have been designed to be biodegradable, and monomer units that are chemical intermediates or products in metabolic pathways have been incorporated. For example, several peptide-based dendrimers, such as those based on polylysine, have been reported [17]. Other examples are the dendrimers of poly(ethylene glycol) (PEG) prepared by Fréchet and co-workers [18] or the polyester dendrimers based on the monomer 2,2-bis(hydroxymethyl)propionic acid and their hybrids with linear PEG [19,20]. Dendritic polymers incorporating glycerol, succinic acid or lactic acid as monomers have been developed by several groups (Fig. 1e) [21–23].

As an alternative to perfect dendrimers, hyperbranched polymers with narrow molecular weight distribution have been introduced by our group and others [24–27]. The anionic ring-opening multibranching polymerization of glycidol resulted in hyperbranched polyglycerol (PG, Fig. 1f) with an inert polyether backbone and functional hydroxyl groups at every terminal unit. This structural feature resembles the well-known poly(ethylene glycol) (PEG) that is accepted for various biomedical applications [28,29]. Dendritic PGs, as characterized by tunable end group functionalities, defined topological 3D architecture, and inertness to non-specific interactions with biological systems, present a novel platform for next generation biomaterials [2,30]. The versatile synthesis allows for a structural range of PG architectures from perfect dendrons to well-defined hyperbranched polymers, megamers, microgels, and hydrogels [31].

Another important development in dendrimer chemistry with interesting biological applications are the cleavable dendrimers that dissemble through the cascade dissociation of covalent bonds. This kind of dendrimer, known as self-immolative, serves as a reservoir of the molecular species which are released with the fragmentation of the dendrimers [32,33].

2. Drug encapsulation and macromolecular prodrugs

In dendrimer-based drug delivery systems, a drug is either non-covalently encapsulated in the interior of the dendrimer or covalently conjugated to form a macromolecular prodrug. Initial studies of dendrimers as potential delivery systems focused on their use as unimolecular micelles and dendritic boxes for the non-covalent encapsulation of bioactive agents [34]. For example, in early studies, DNA was complexed with PAMAM dendrimers for gene delivery applications [35] and hydrophobic drugs and dye molecules were incorporated into various dendritic cores [36,37]. A major drawback of these delivery systems is the lack of controlled drug release kinetics, because most systems release their payload over the course of several hours. In some cases, harsh conditions are required, whereas in others the encapsulated drug is not well retained and the molecules are released faster [15,38].

The introduction of poly(ethylene glycol) PEG chains on the dendrimer periphery has expanded the scope of dendritic unimolecular micelles as drug carriers because it allows a better control over drug release rate and improved biocompatibility.

An alternative approach in the development of dendrimers as drug delivery carriers is to exploit their well-defined multivalency for the covalent attachment of drug molecules. Dendrimer/drug conjugates generally consist of a therapeutic agent covalently linked to the peripheral groups of the dendrimer. This method offers distinct advantages over physically encapsulated systems. Multiple drug molecules can be attached to each dendrimer and the release of these therapeutic agents is partially controlled by the nature of the linkages. The drug loading can be tuned by varying the generation number of the dendrimer, and release kinetics can be controlled by incorporating degradable linkages between the drug and the dendrimer. As example, Duncan and co-workers [39,40] have prepared conjugates of PAMAM dendrimers with cisplatin, a potent anticancer drug with non-specific toxicity and poor water solubility. The conjugates show increased solubility, decreased systemic toxicity, and selective accumulation in solid tumors.

3. Stimuli-responsive nanocarriers

In contrast to simple complexes or conjugates, stimuli-responsive materials can undergo relatively large and abrupt physical or chemical changes in sharp response to external stimuli in the environmental conditions. Some of the stimuli are physical, as temperature, ionic strength, light, solvents, strength of magnetic or electrical field while others are chemical/biochemical in origin like pH-shift, redox microenvironment, enzyme over-expression, host–guest recognitions or antigen–antibody interactions, etc. [41].

The use of stimuli-responsive nanocarriers offers an interesting opportunity for drug and gene delivery where the delivery system becomes an active participant, rather than passive vehicle, in the optimization of therapy. The benefit of stimuli-responsive nanocarriers is especially important when the stimuli are a characteristic component of disease pathology (specific enzyme, protein over-expression, pH, electrolyte status etc.). This allows the nanocarrier to respond specifically to the pathological triggers and substantially reduce the side-effects [42,43].

The general concept of triggered release, as shown in Fig. 2, can be divided into two major modes according to the nature of the interaction between the bioactive molecule and the dendritic polymer. In the encapsulation approach (Fig. 2a), the release can be triggered by structural change within the polymeric scaffold (i.e., backbone degradation, cleavage of shell, charging of functional groups, etc.) while in the macromolecular prodrug approach, the mechanism of release involves the splitting of the linker between the polymer and the bioactive agent (Fig. 2b).

Several chemical linkers which respond to external stimuli have been reported (Fig. 3) [8]. In the following sections we present different concepts of environment sensitive dendritic scaffolds for delivery of bioactive agents. The purpose of this review is to highlight different concepts of environment sensitive dendritic scaffolds for delivery of bioactive agents rather than extensively covering the entire field of stimuli-responsive materials. For further details readers are referred to specific references [3,44–47].

3.1. pH-responsive systems

The pH condition of pathological tissues, such as inflammation, infection, and cancer, is significantly different from normal tissues. In particular, the extracellular and intracellular pH profile of the biological system is greatly affected by diseases. For instance, the extracellular pH in solid tumors tends to be significantly more acidic (6.5) than the pH of the blood (7.4) at 37 °C [48–52]. In addition, the pH values of endosomal and lysosomal vesicles inside the cells are also significantly lower than cytosolic pH. These differences in pH could allow the delivery of the payload by a simple protonation of the dendritic structure or by the cleavage of acid-labile moieties, which can specifically occur in specific extracellular or intracellular compartments.

The simplest approach for pH triggered release of encapsulated guest molecules involves the protonation of certain functional groups at the core or surface of the dendritic architecture. Several examples have been published using amine or carboxylic acids as the most common functional groups [53–58]. The release of pyrene encapsulated into the fourth and fifth generation of PPI-dendrimers (DAB32 and DAB64 respectively) has been reported and measured via fluorescence intensity in water [59]. The system could be fine-tuned via addition of quaternary ammonium groups that seal the surface to release the encapsulated pyrene in a narrower pH region [60].

Vögtle et al. reported the development of systems for pH-responsive encapsulation and release of guest molecules [61].

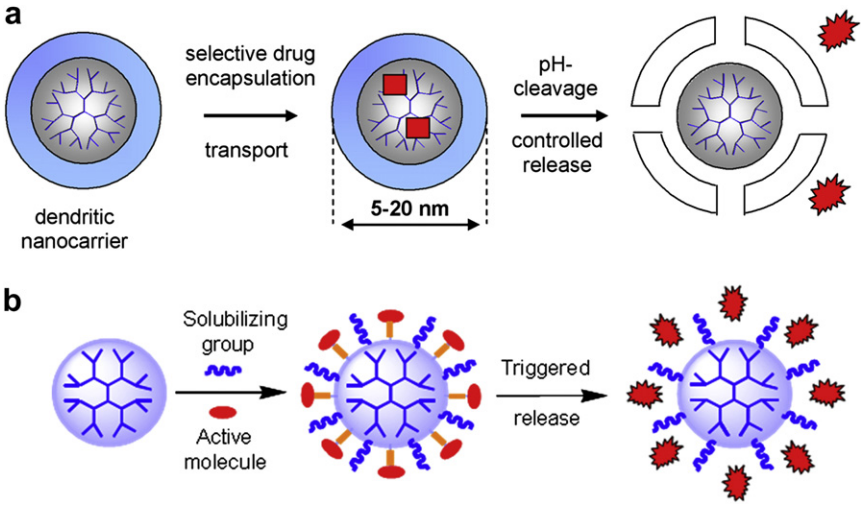


Fig. 2. Different mechanisms for stimuli-responsive release of bioactive molecules: (a) from non-covalent complexes (b) from covalently conjugated architectures.

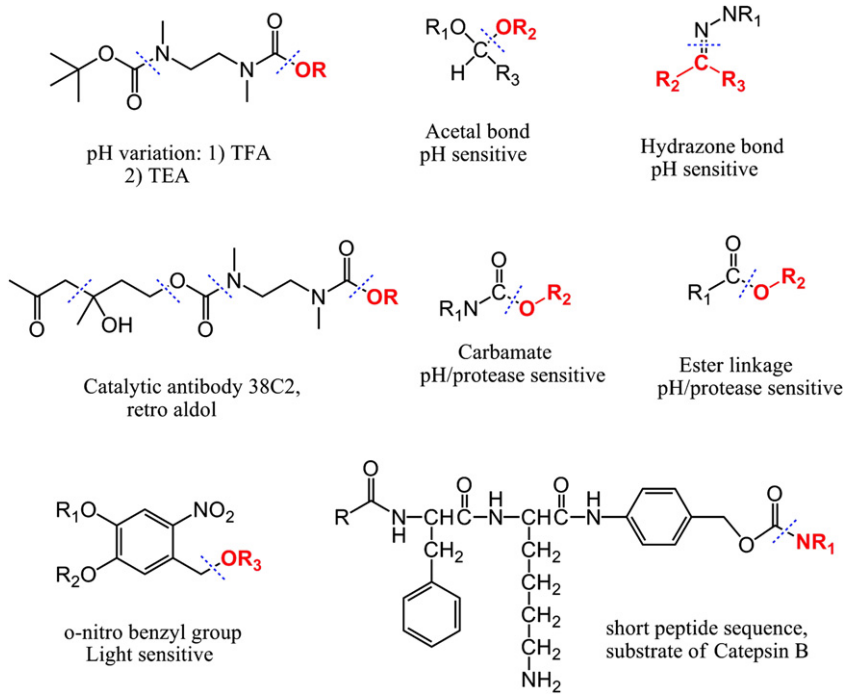


Fig. 3. Chemical entities involved in stimuli-responsive cleavage. The dashed lines indicate the bonds which are cleaved.

They described the use of lipophilic urea functionalized PPI-dendrimers which are able to encapsulate the diagnostically relevant anions pertechnetate, perrhenate, and adenosine-5′-triphosphate (ATP) [62]. A lower pH results in a higher extractability of the functionalized dendrimers due to protonation of the amine groups of the dendrimer.

Smith and co-workers reported the synthesis of degradable dendrons for high-affinity DNA binding promoted by spermine moieties [63]. The dendrons were constructed from repeated ester groups, with the oligoamines being attached to the surface through carbamate modalities. Both of these linkages showed potential chemical biodegradability which enabled degradation under biological conditions and allowed further release of the gene fragments.

An alternative mechanism for triggering the release in acidic environment is through conformational changes of supramolecular aggregates, e.g., by partial degradation of the structural subunits. As an example, PEG-dendrimer hybrids used as backbones for acid-sensitive micelles have been investigated by attaching PEG to either a polylysine or polyester dendron. This approach involves the incorporation of hydrophobic groups to the periphery of the core-forming dendrimer block using an acid-sensitive acetal linkage. Upon hydrolysis of the linkage, the core-forming block becomes hydrophilic, thus destabilizing the micelle and allowing the release of the drug from its encapsulating micellar compartment [64].

An extensively studied pathway is the disabling of core-shell structures by cleavage of the shell. The first pH-responsive dendrimer reported with this feature was the so-called “dendritic box” [37]. With this system it was possible to selectively release encapsulated guest molecules after partial or total cleavage of the shell. The hydrophilic core used was a fifth generation poly (propylene imine) dendrimer (G5-PPI-dendrimer), which was able to encapsulate two guest molecules (rose bengal and p-nitrobenzoic acid) after attachment of a BOC protected amine acid shell. The partial cleavage of the shell with formic acid leads the removal of the BOC groups, which allowed the release of the small guest molecule p-nitrobenzoic acid. Upon cleavage of the amide bond with HCl, rose bengal could also be liberated. Although this is a very impressive example, the release of the encapsulated guest molecules takes place under relatively harsh conditions at elevated temperatures and is therefore not suitable for drug release in the body. In addition, the molecule is only soluble in organic solvents.

Based on a similar concept, a simple and general method for the generation of core-shell-type architectures from readily accessible hyperbranched polymers was extensively explored by our group [65–68]. Several pH-sensitive nanocarriers have been prepared by attaching pH-sensitive shells through acetal or imine bonds to commercially available dendritic core structures, such as polyglycerol and polyethylene imine. In some cases the pH-responsive nanocarriers showed a very high transport capacity which is an important criterion for efficient drug delivery. Various guest molecules, such as polar dyes, oligonucleotides, and anticancer drugs have been encapsulated inside these dendritic core-shell architectures. Furthermore, an optimal release behavior was observed: fast release at pH 5–6 and slow release at pH 7.4. Among them were several water-soluble systems which localize in tumors in vivo as demonstrated by fluorescence imaging in tumor-bearing mice with indotricarbocyanine–nanocarrier complex [65].

The macromolecular approach has been shown to be promising for the smart delivery and release of drugs under acidic environments. The direct coupling of active drugs to dendritic scaffolds using pH-labile linker, such as acetal or hydrazone, has already shown high efficacy within in vitro and in vivo tumor models. The antitumor effect of doxorubicin conjugated through a hydrazone linkage to an asymmetric, biodegradable polyester dendrimer termed as ‘bow-tie dendrimer’ was evaluated in mice bearing C-26 colon carcinomas. In culture, the dendrimer/doxorubicin prodrug was 10 times less toxic than free doxorubicin towards C-26 colon carcinoma cells. In efficacy studies, a single i.v. injection of dendrimer-doxorubicin prodrugs at 20 mg/kg doxorubicin equivalents caused complete tumor regression and 100% survival of the mice over the 60 days experiment [69].

In an ongoing investigation in our group, we prepared a series of conjugates of hyperbranched polyglycerol with (6-maleimidocaproyl) hydrazone derivative of doxorubicin (DOXO–EMCH). The conjugates showed an acid-triggered release at pH 5.0 while only a marginal release was observed at pH 7.4. The antiproliferative activity assessed against two human tumor cell lines, i.e., AsPC1 LN (pancreatic carcinoma) and MDA-MB-231 LN (mamma carcinoma), showed a 2- to 10-fold lower cytotoxicity than the corresponding free drugs. With respect to antitumor efficacy, the conjugates manifested excellent antitumor effect with complete tumor remission up to day 30 without significant changes in body weight, even after administration of 3-fold the maximal tolerated dose for free doxorubicin [70,71].

3.2. Redox microenvironment

As mentioned before, the pathological scenario in damaged tissues presents particular characteristics which may enable the selective release of bioactive molecules at the site of action. The differences in redox potential as well as the over-expression of certain enzymes at cellular level have been extensively used as triggering signals.

The distinct difference in redox potential (100–1000 fold) existing between the reducing intracellular space and the oxidizing extracellular space is a potential stimuli for the triggered release of therapeutics agents. Dendritic delivery systems containing disulfide linkages may undergo disulfide cleavage in lysosomal compartments. The glutathione pathway which controls the intracellular redox potential [72] is significantly involved in such stimuli-sensitive mechanism. In this concept, a drug or gene fragment can be encapsulated or conjugated to dendritic nanocarriers carrying disulfide bonds. Once the disulfide bonds are reduced in the presence of excess of glutathione inside the cell, the drug or gene present in the nanocarrier is released.

Several examples in the dissociation of complexes after reduction of disulfide linkage located within the dendritic structure have been reported in the field of gene delivery. Crosslinked poly (ethylene imine) (PEI) polyplexes for intracellular DNA release were generated using the low molecular weight crosslinking reagent dithiobis(succinimidyl propionate). The disulfide bonds of the crosslinked polymer/DNA complexes (polyplexes) proved to be susceptible to intracellular redox conditions, allowing the release of DNA after cleavage of the disulfide bonds. Cell culture experiments under reducing conditions as well as with glutathione loaded cells confirmed the proposed intracellular activation and the efficient DNA transfection [73].

Kostiainen et al. reported the synthesis of polylysine dendrons with multiple spermine groups attached to the dendritic scaffold through disulfide linkers. The controlled breakdown of the multivalent dendritic surface allowed the release of the covalently attached spermine and the non-covalently attached DNA. The resulting individual spermine groups have only a weak affinity towards DNA, which was therefore effectively released [74].

Our group has recently developed a method for the preparation of polyglycerol microgels which can achieve post-endocytotic nanoparticle cleavage by using redox labile disulfide bonds in the polymer backbone [75]. These PG-microgels are nanoparticulate systems with characteristics of both hyperbranched polymers and macroscopically crosslinked materials. Their privileged nanosized range of 20–100 nm is useful for optimal cellular uptake and its concomitant activity as drug nanocarrier [76,77]. The potential limitation on their application, related to tissue and organ accumulation, has been overcome through the use of disulfide linkers within the polymeric architecture. An efficient release of encapsulated indocarbocyanine (ICC) dye was confirmed after reductive degradation of the microgels into smaller fragments, mediated by the addition of the reducing agent glutathione.

Cysteine and analogues thereof have always been the 'fragment of choice' for designing environment sensitive dendritic architecture. Kono and co-workers have investigated the structural sensitivity of dendrimers against oxidative and reductive environment using cysteine. They demonstrated that PEG-attached PAMAM G4 dendrimer that had cysteine residues manifested environment sensitive associating property against rose bengal. The disulfide linkages of the cysteine residues located at the periphery of the dendrimer acted as an effective barrier which controlled the access of the small molecules into the dendrimer interior [78].

Also polymer conjugates between PAMAM dendrimer and N-acetyl-L-cysteine (NAC) were reported as a macromolecular prodrug which takes advantage of the high intracellular glutathione levels [79]. Two conjugates were successfully synthesized with a cationic G4-PAMAM-NH_2 and an anionic G3.5-PAMAM-COOH dendrimer with NAC payloads of 16 and 18 per dendrimer, respectively. Effective release of the NAC linked through a disulfide linkage was confirmed at intracellular glutathione level ($\sim$10 mM), whereas negligible NAC release was observed at extracellular GSH levels (2 μM).

An example in the area of diagnostics described macromolecular Gd (III) chelates, which are superior magnetic resonance imaging (MRI) contrast agents for blood pool and tumor imaging. The stimuli-responsive approach can represent a solution to overcome the safety concerns related to the slow excretion and long-term gadolinium tissue accumulation. A sixth generation PAMAM-Gd(III) chelate conjugate with a cleavable disulfide spacer was prepared as a biodegradable macromolecular MRI contrast agent with rapid excretion from the body [80]. Blood pool and tumor contrast enhancement of the agent were evaluated in female nude mice bearing MDA-MB-231 human breast carcinoma xenografts with a nondegradable conjugate as a control. The cleavable system resulted in a significant contrast enhancement in the blood for about 5 min, a more prominent tumor contrast enhancement, and an optimal release profile of Gd followed by a rapid excretion via renal filtration after the disulfide spacer was cleaved. The nondegradable control had much longer blood circulation and excreted more slowly from the body.

3.3. Enzymatic stimuli

Enzyme-triggered drug release has also attracted much interest [81]. Common functional groups used for this purpose are ester derived moieties and short polypeptide sequences, which are substrates for several enzymes over-expressed in malignant tissues [82,83].

Following this concept, an effective approach was reported by our group for the preparation of gene carriers, where oligoamine moieties (spermidine, spermine and pentaethylenhexamine) were conjugated to hyperbranched polyglycerol using a carbamate linkage [84,85]. The PG based nanocarriers proved to be quite effective for the condensation and cytoplasmic release of siRNA, as was demonstrated by the high silencing efficacy of the polyplexes.

In the macromolecular prodrug approach, the drugs are conjugated to its carrier via a chemical bond which is cleavable by a specific enzyme present in the target site. As example, naproxen was conjugated to a PAMAM dendrimer as a model for poorly water-soluble drugs to enhance drug solubility and bioavailability for oral delivery applications. Conjugation was carried out either directly via an amide bond or via ester bonds using L-lactic acid or diethylene glycol as linker molecules. pH-dependent studies revealed that amide and ester linkages were stable in buffer solutions in a broad range of pH. However, naproxen was enzymatically

released from both ester conjugates in 80% human plasma. The lactide ester hydrolyzed slowly releasing about 25% of naproxen within 24 h, while the diethylene glycol ester hydrolyzed quickly ($t_{1/2}$ = 51 min). Permeability studies across Caco-2 monolayers at 37 °C showed significant enhancement in the transport of naproxen-dendrimer prodrugs [86,87].

Baker and co-workers have extensively explored the conjugation of anticancer drugs to PAMAM dendrimers mediated by ester groups. Methotrexate was conjugated via an ester bond to the fifth generation PAMAM dendrimers with partially acetylated amine surface. The remaining amino groups were utilized to conjugate folic acid (FA) via an amide linkage as an active targeting ligand and the dye fluorescein isothiocyanate (FITC) via a thiourea linkage as an imaging moiety to the dendrimer surface. The dendritic conjugate was studied in vitro using folic acid receptor-expressing KB cancer cells, and a time- and dose-dependent inhibition of cell growth was observed. In an attempt to verify the in vitro results, the conjugates were injected i.v. into immunodeficient nude mice bearing human KB tumor xenografts and showed improved in vivo efficacy and biodistribution profile [88]. Similar studies were carried out using paclitaxel as a model drug which was conjugated through its hydroxyl group at 20-position to the same PAMAM dendritic carrier as methotrexate [89].

Minko et al. synthesized a polyamidoamine (PAMAM) dendrimer-succinic acid-paclitaxel conjugate with an ester bond which could be cleaved by esterase hydrolyzing enzyme [90]. In vitro data showed that cytotoxicity increased 10-fold using the conjugate form compared to the free unconjugated drug. These dendrimer prodrugs provided both cytoplasmic and nuclear delivery of therapeutics and enhanced anticancer activity of paclitaxel.

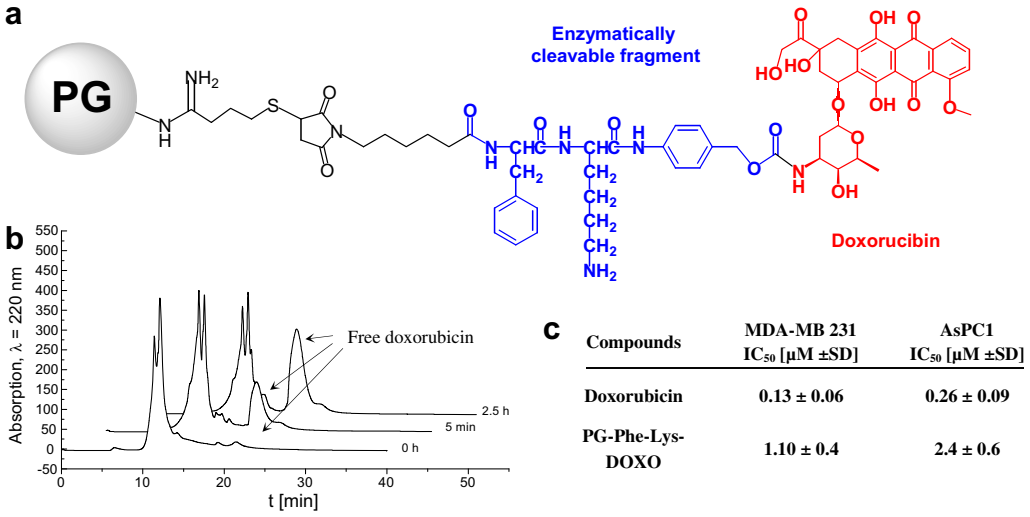
Shabat and co-workers developed a novel prodrug platform of 'self-immolative' dendrimers [91–94]. These uniquely structured dendrimers can release all of their tail units through 'self-immolative' chain fragmentation, which is initiated by a single cleavage event at the dendrimer core. Incorporation of drug molecules (doxorubicin, camptothecin, and etoposide) as the tail units and an enzyme substrate as the trigger can generate a multi-prodrug unit that becomes activated upon a single enzymatic cleavage. Evaluation of the system using a cell-growth inhibition assay showed that the heterotrimeric prodrug had a potency increment of 15-fold upon activation by antibody 38C2. A similar approach of controlled dendrimer dissociation was applied to enhance the activity of a dendritic fluorescence probe [95].

In a recent communication, we reported the use of the thiolated hyperbranched polyglycerol scaffold for conjugation to maleimide-bearing prodrugs of doxorubicin or methotrexate which incorporate either a self-immolative para-aminobenzyloxycarbonyl (PABC) spacer coupled to dipeptide Phe-Lys or the tripeptide D-Ala-Phe-Lys as the protease substrate [96]. Both prodrugs were cleaved by cathepsin B, an enzyme over-expressed by several solid tumors, to release doxorubicin or a methotrexate lysine derivate (Fig. 4). Cytotoxicity of the conjugates against human tumor cell lines showed that the activity of the drugs was primarily retained, which confirmed the macromolecular prodrug concept.

3.4. Photo-sensitive systems

Utilization of light as an external stimulus offers a range of advantages, including ease of application, relative biocompatibility and controllability in both spatial and temporal perspective [97–101]. The principle of photo-responsive dendritic architectures relies on the adjustable release of encapsulated/conjugated bioactive units from the structure under the influence of light of specific frequency. The earlier experiments on photo-responsiveness of dendritic architectures have been reported by McGrath et al. [32,102]. For this purpose, they utilized photolabile o-nitrobenzyl ether moiety. Since then, extensive research has been performed in the field of photo-responsive dendrimers and hyperbranched polymers. They are mainly focused towards a photo-induced conformational change of the responsive units that leads to cross-linking or cleavage of the entire structure. Although a substantial amount of research on photo-sensitive dendritic architectures has been mainly directed to the field of opto-electronics and surface modification [103], the potential of such systems is also of significance in the fields of biology and medicine.

Kim et al. reported the self-assembly of amide dendrons with a photo-responsive focal functionality, which releases entrapped molecules upon exposure to UV light. The photocleavable 2-nitrobenzyl ester moiety and photoisomerizable azobenzene unit were introduced at the focal point of the amide dendron to construct supramolecular structures with a photoswitchable function (Fig. 5) [104].



Compounds	MDA-MB 231 IC_{50} [μM ±SD]	AsPC1 IC_{50} [μM ±SD]
Doxorubicin	0.13 ± 0.06	0.26 ± 0.09
PG-Phe-Lys-DOXO	1.10 ± 0.4	2.4 ± 0.6

Fig. 4. Enzymatic cleavable prodrugs derived from dendritic polyglycerol. (a) Schematic representation of doxorubicin prodrug. (b) Size-exclusion chromatogram of an incubation study of PG-Phe-Lys-DOXO at pH 5.0 and 37 °C with cathepsin B. The cleavage product doxorubicin elutes at ~20 min. (c) IC_{50} values of doxorubicin and PG-Phe-Lys-DOXO against the human tumor cell lines MDA-MB-231 and AsPC1.

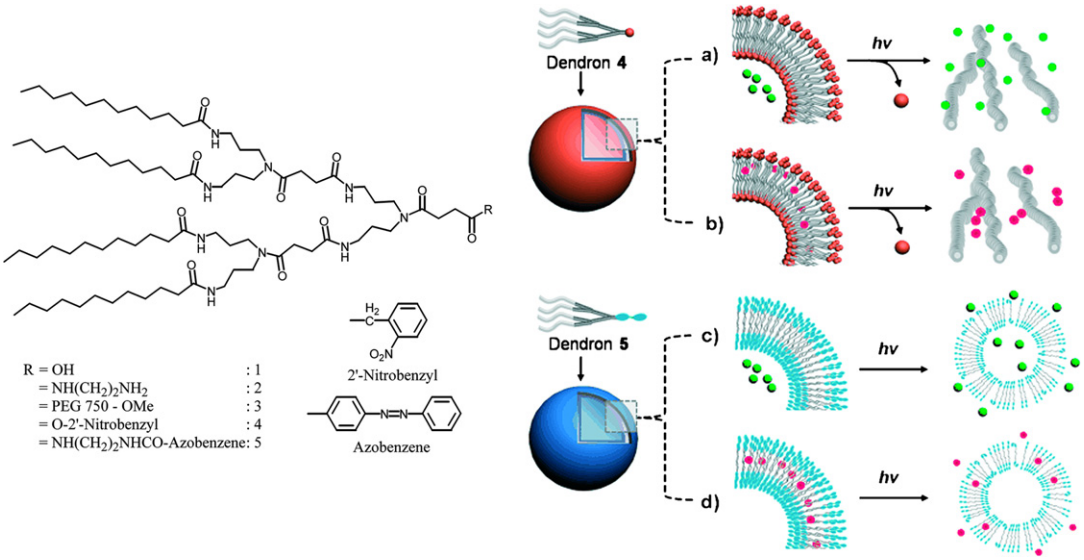


Fig. 5. Schematic illustration for the release characteristics of the vesicles upon irradiation with UV light. Green sphere: calcein, red: nile red. Reproduced with permission from [104]. Copyright 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

A convenient methodology for the synthesis of photolabile, crosslinked hyperbranched polyglycerol nanocapsules has been reported [105]. These nanocarriers selectively and efficiently bind ionic guest molecules. The stability of the host–guest complexes formed depends on the counterion of the guest molecules. Moreover, the control over guest binding can be achieved by modification of the polymer building blocks, in particular the outer shell. In addition, photo-triggered degradation of the nanocarrier leads to an efficient release of encapsulated guest molecules.

Smith and co-workers have generated the dendritic structures as illustrated in Fig. 6 [106]. The figure illustrates the use of the cleavable shells for release of gene fragments following the approach as described in references [73] and [83]. The dendrons are modified by attaching spermine surface groups through an o-nitrobenzyl linker. The o-nitrobenzyl group undergoes photolytic degradation when submitted to long-wavelength UV light (l = 350 nm), thus allowing a controlled release of the covalently attached spermine surface groups and the non-covalently bound DNA.

Combination of pH-sensitive modalities with a photo-responsive dendritic system is of importance for constructing smart materials with interesting properties. Moroder et al. synthesized dendritic peptides with branched lysine core containing eight azobenzene moieties in the periphery [107]. With an additional peptidic tail consisting of an oligolysine portion, water solubility was achieved for the dendrimers, which enabled the cis/trans photoisomerization of the dendritic azobenzene species in both organic and aqueous media.

Photo-responsive self-immolative dendron (SID), as reported by Shabat and co-workers, is an interesting example of the light-triggered activation of a dendritic platform in general. These dendrons release their end-groups through a photo-triggered self-immolative process initiated by a single photocleavage at a focal point [108].

3.5. Thermo-responsive dendritic architectures

Dendrimers containing thermo-responsive units are advantageous for constructing molecular architectures which reversibly respond to change of temperature. In principle, the change with temperature occurs within a narrow range [109,110].

After the first report of McElhanon et al. on reversible thermolysis of dendrimers, an extensive amount of research has been carried out in this field [111]. As an example, a novel terminal modification agent to endow hyperbranched polyamidoamine (HPAMAM) with thermo-/pH-responsive properties was reported by Huang et al. [112]. HPAMAM with terminal vinyl groups was first synthesized and then end-capped by 1-adamantylamine (ADA). The resulting hyperbranched polymer (HPAMAM-ADA) showed interesting thermo-responsive properties in aqueous solution. The lower critical solution temperature can be controlled by adjusting the end-capping ratio of ADA. In addition, HPAMAM-ADA exhibits a pH-dependent water solubility.

For designing thermo-responsive camptothecin nanocarrier, a series of dendritic polyamidoamine-polyethylene glycol-poly(D,L-lactide) (PEG–PDLLA) constructs were synthesized through conjugation of PEG of various chain lengths to the third generation of PAMAM dendrimer with subsequent ring-opening polymerization of DLLA. It was found that dendritic PEG–PDLLA nanoparticles in aqueous solution can self-assemble into sub-micron/micron aggregates, the size of which is dependent on temperature and PEG–PDLLA chain length. The potential of dendritic PEG–PDLLA nanoparticles for encapsulation of water-insoluble drugs such as camptothecin was demonstrated. The constructed dendritic PEG–PDLLA nanoparticles possessed high cytocompatibility in HN12 cell lines, which was significantly improved compared with PAMAM dendrimers [113].

Kono and co-workers have prepared PAMAM G4 dendrimers with n-butyramide, isobutyramide and cyclopropanecarboxylic acid amide groups at the dendritic chain end by the reaction of amine-terminated PAMAM G4 dendrimer with n-butyric acid, isobutyric acid, and cyclopropanecarboxylic acid. Their systematic investigation led to a generalized observation that surface modification with alkylamide groups which are common structural units with well-known thermo-responsive polymers with a linear structure, provides temperature-sensitive properties to dendritic PAMAM scaffold.

The same group has reported the preparation of hyperbranched polyglycerol with NIPAM moieties that imparted thermosensitivity and pH-responsiveness to the PG scaffold in ranges around normal physiological conditions [114]. A combination of

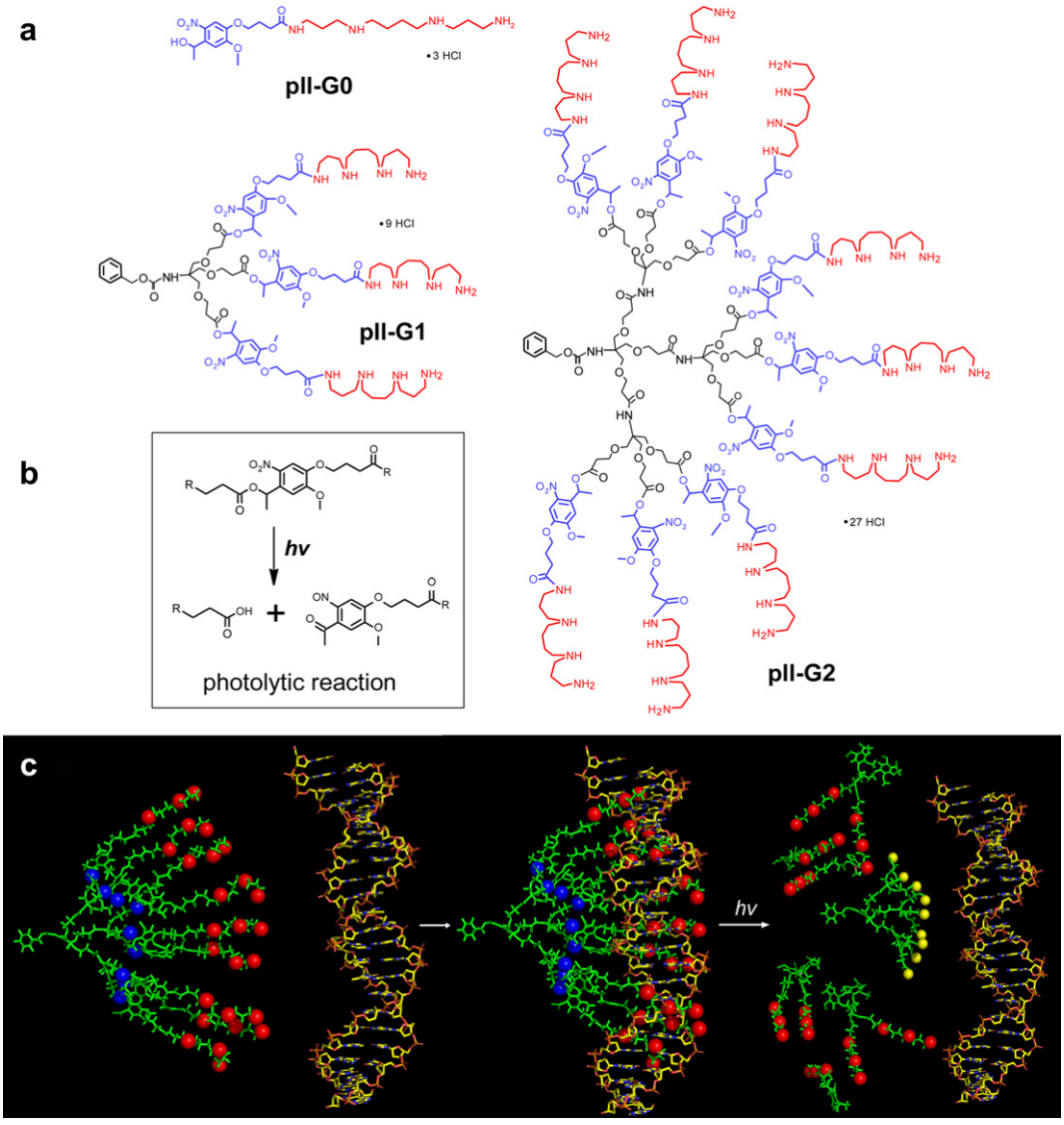


Fig. 6. Spermine derivatives. a) Target photolabile dendrons pll-G0, pll-G1, and pll-G2. b) The photolysis of pll-G1 and pll-G2 liberates the spermine surface and exposes the carboxylic acids. c) Self-assembly of multivalent dendrons and DNA, and subsequent optically triggered degradation of the cationic surface and the release of DNA. Blue spheres: photocleavage sites, red spheres: cationic spermine amines, yellow spheres: anionic carboxylic acid groups exposed after photolysis. Reproduced with permission from [107]. Copyright 2007 Wiley-VCH Verlag GmbH & Co. KGaA.

pH (in the range of 5–7) and temperature influence on thermosensitivity of the PG scaffold confirmed the stimuli sensitivity imparted upon the structure by succinic acid and NIPAM units. The thermosensitive compound showed that it could potentially act as a nanocapsule for carrying/releasing bioactive molecules, as demonstrated with rose bengal as model compound. Similar methodology was used for the preparation of stimuli-responsive gold nanoparticle assemblies by encapsulation of the nanoparticles within NIPAM-HPG polyelectrolytes, leading to a sharp phase transition upon change of temperature or pH value [115].

4. Conclusions

With the emergence of nanomedicine, the need for smart and innovative polymeric materials has become quite critical. In particular, encapsulation, transport, and selective release of active compounds on the molecular level are very important for administering cytotoxic or unstable bioactive compounds, e.g., antitumor drugs, imaging probes, DNA, or siRNA. The release of these active molecules near the vicinity of the targeted tissue is essential for maximizing clinical efficacy. Coupling the advantages of dendritic nanocarriers with stimuli-responsive modalities, as reviewed in this article, shows a new generation of smart materials that can be designed for achieving optimal therapeutic benefit. However, many designer systems are still lacking an in vivo proof of concept.

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