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REVIEW

A review on synthesis and applications of dendrimers

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Abstract
Among molecular structures, colloidal molecules have attracted the attention of the scientific community because of their distinct geometry (topology, size on a nanometer scale). In this field, dendrimers are an example of colloidal polymeric molecules which have a specific size and are identified and regulated by repeating units as generations. The dendrimers are very similar to the colloidal particles in geometric form and size, which is more evident in the higher generations of the colloidal dendrimers. On the other hand, the unique structural features of these macromolecules resemble them as macromolecular colloids. These properties including the high degree of freedom, the controllable molecular size and weight, the need for no initiator, the presence of end groups, the high drug transfer capacity, etc., have made dendrimers applicable to a variety of fields such as medicine, biomedical, pharmaceutical, catalyst, and so on.

Keywords Dendrimer · Colloid · Synthesis · Bioapplications

Abbreviations

PPI	Poly(propylene imine) dendrimer
PAMAM	Poly(amidoamine) dendrimer
LC	Liquid crystalline dendrimer
Tecto	Core–shell dendrimer
MRI	Magnetic resonance imaging
EDA	Ethylenediamine
DAB	1,4-Diaminobutane
DOX	Doxorubicin
PLL	Poly(L-lysine) dendrimers
Tf	Transferrin
G	Generation
PEG	Polyethylene glycol
AuNPs	Gold nanoparticles
Cr	Chromium
DENs	Dendrimer nanoparticles
Pt	Platinum
Cu	Copper
Pd	Palladium
3D	3-Dimentional
MCF-7	Breast cancer
A549	Adenocarcinomic human alveolar basal epithelial cells
BCSFB	Blood–cerebrospinal fluid barrier
BBB	Blood–brain barrier
Zn	Zinc
Fe	Iron
CT	Computed tomography
SM	Single molecule

Introduction

Dendrimers as globular nano-macromolecules are radially symmetric molecules with well-specified, uniform and integrated structure including tree-like arms or branches [1–4]. The structure of these materials has a profound influence on their physical and chemical properties. Due to their unique behaviors, they are used in a broad range of biomedical and industrial applications. Unbeatable features of dendrimer such as the same size, the highest degree of branch formation, water solubility and presence of internal cavities make them attractive for various applications [5, 6]. In addition, some dendrimers have natural medicinal activities such as antibacterial, antiviral and antitumor activities [7].

Although research on dendrimers began in 1970s, no successful method was reported by the late 1970s. In

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1978, Buhleier et al. [5] introduced a group of synthetic cascading molecules. In 1984–1985, Tomalia et al. [7] reported the first real dendrimers, and in 1990, Hawker et al. [8] published a report on preparation of spherical molecules. Unlike linear polymers, these macromolecules do not entangle [9, 10] and have unusual viscosity behaviors such as low solution viscosity and functional groups can be either protected or exposed [11, 12]. These unique macromolecules are made up of three basic parts, namely a central core with at least two identical chemical functions, branches exuding from the core which are divided into several layers called generations and terminal groups which have important role in properties of dendrimers [13, 14].

Synthesis approaches

Divergent method

In this approach, dendrimer begins to grow from a multifunctional core through a step-by-step iterative addition of monomers [15, 16]. The advantages of this approach are the high rate of polymerization, modification and change in surface groups, and synthesis of high molecular weight dendrimers [17, 18]. However, side reactions occurring during synthesis are the most important disadvantage of this approach, especially in poly(amidoamine) dendrimers. Also, incomplete reactions occur in the end groups and lead to structural defects [19]. Other disadvantages of this approach include formation of some low molecular weight molecules, lack of diversity in group's outer layers and high reaction temperature sensitivity which can cause reversible Michael addition reaction [20].

Convergent approach

Convergent approach was introduced to overcome the disadvantages of divergent approach [20]. In this approach, highly pure dendrons are produced firstly and then connected by a multifunctional core. This approach has several advantages including easy product purification and significant reduction in structural defect. Convergent approach suffers from high number of steps, difficulty in the synthesis of dendrimers with higher generations and reduced returns due to reduced reactivity of the central dendrons [15]. In convergent approach, the formation of higher generations is so difficult due to the occurrence of spatial inhibition in the reaction between dendrons and molecular nucleus [4]. Scheme 1 schematically shows the synthetic path for divergent and convergent approaches.

Click chemistry

Click chemistry approach is another method for the rapid and reliable synthesis of dendrimers. One of the salient features of this method is the high chemical performance of the reaction. Also, simple reaction conditions, readily available reagents, and benign used solvents are other features of this method [21].

Lego chemistry

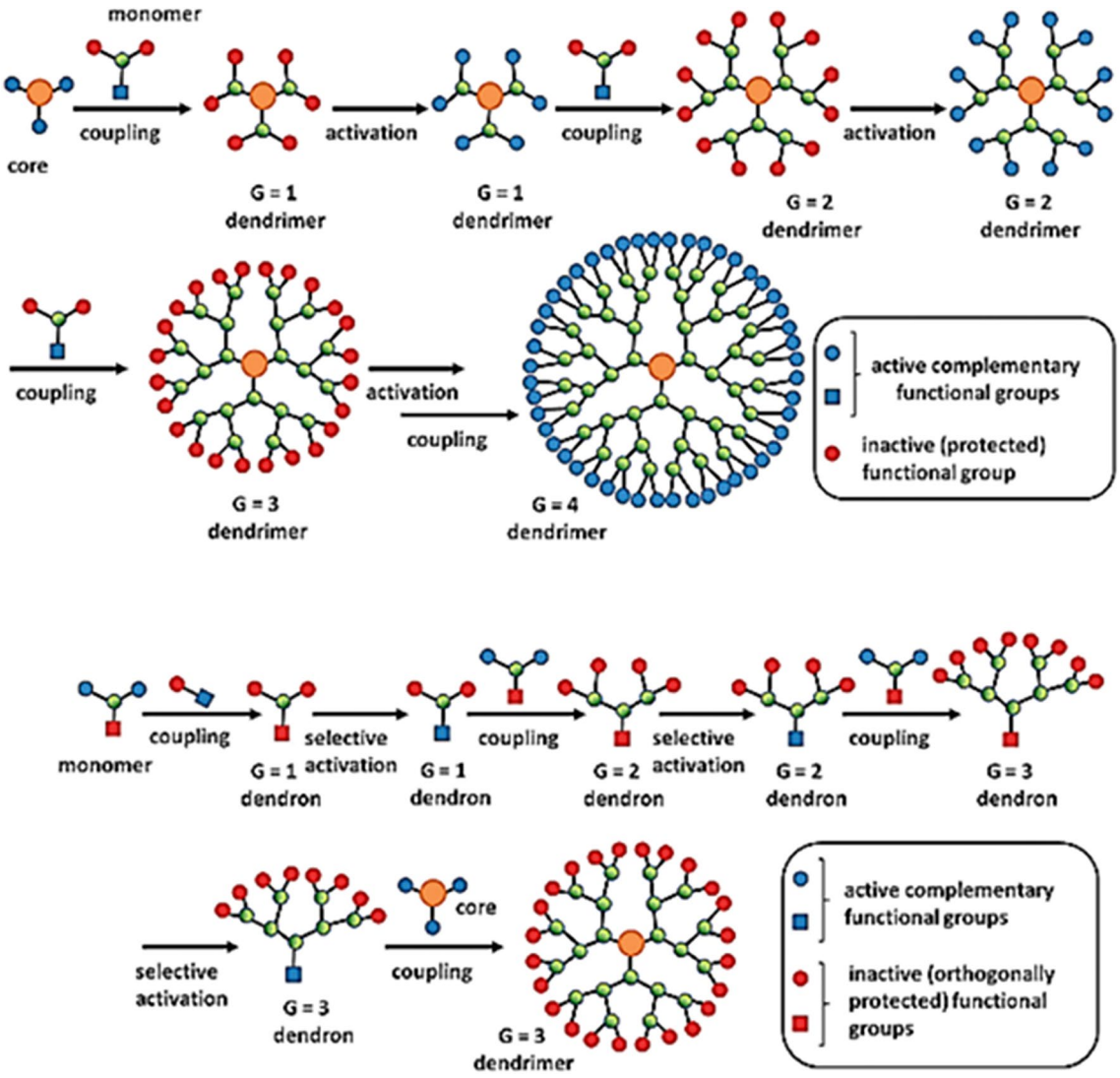
Various methods have been discovered by scientists in order to simplify the synthetic method for the synthesis of dendrimers in terms of cost and synthesis time. Lego chemistry is one of the results of these excavations. This method has been reviewed several times, and the results showed that this method can increase the number of terminal groups from 48 to 250 in one step [21].

Types of dendrimers

Dendrimers based on their shape, peripheral groups and inner cavity can be divided into several types including PAMAM dendrimer [22], PPI dendrimer [23], liquid crystalline (LC) dendrimers [24], chiral dendrimers [25], peptide dendrimers [26], core–shell (tecto)dendrimers [27], glycodendrimers [28], etc. Herein, three types of dendrimers as the most important ones have been widely reviewed.

PAMAM dendrimers

The first successful effort to create and design dendritic structures by organic synthesis was made by Vogtle and colleagues [5]. Then, Tomalia and colleagues succeeded in synthesis of PAMAM dendrimer in the early 1980s [7]. PAMAM dendrimers owing to the combination of superficial amines and interior amide bonds are used in many biological applications [29]. Ease of use, low-cost synthesis of doped PAMAM dendrimers than other biological molecules such as antibodies and proteins of the same size and biocompatibility have made them interesting for biochemistry, nanotechnology and medicine [29–31]. These dendrimers are generally synthesized via divergent approach [5]. Core molecules which give rise to PAMAM dendrimers can vary but the most basic initiators are ammonia [32] and ethylenediamine [33]. As shown in Scheme 2, PAMAM dendrimers use the following iterative reactions to grow:



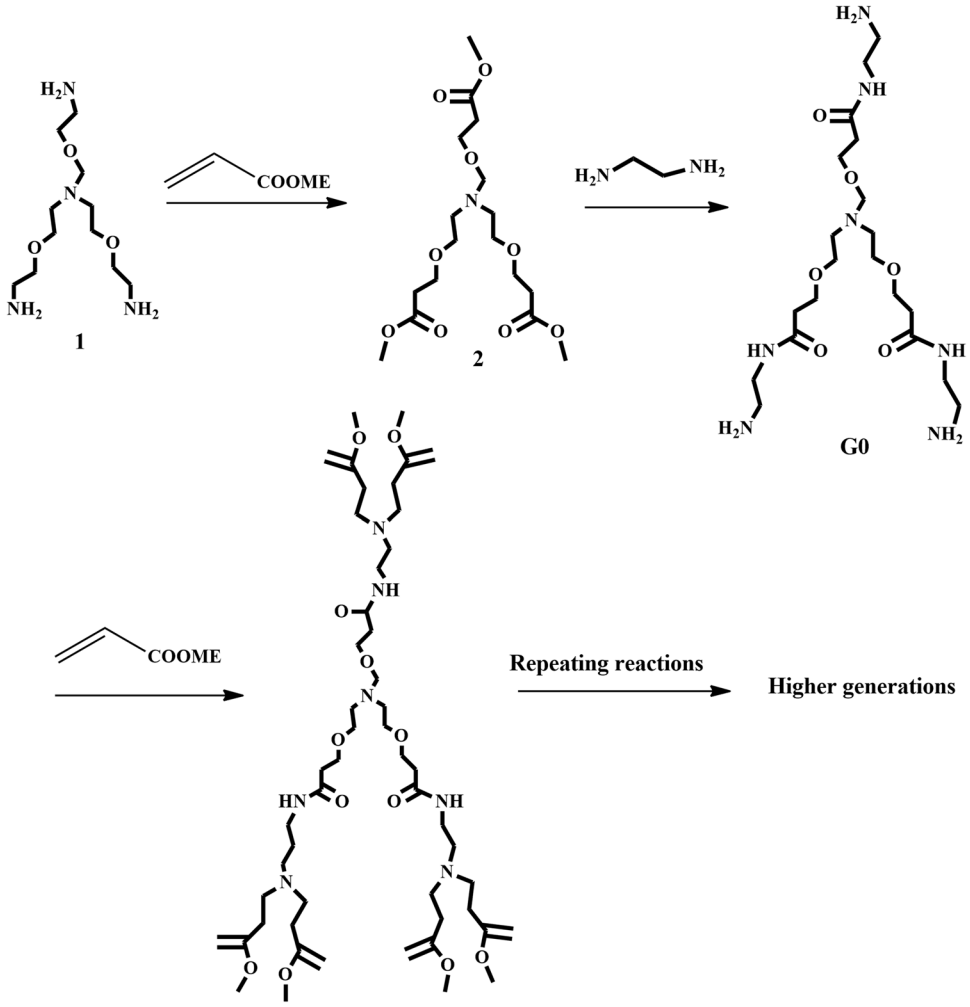
Scheme 1 Synthetic path for divergent and convergent approaches Reproduced with the permission of RSC, 2020 [19]

(1) Michael addition of the amino-terminated compound onto methyl(meth)acrylate;
(2) Amidation reaction between amine-containing compounds and product of stage (i) to achieve a new amino-terminated compound.

It should be noted that focal points of convergent-synthesized segment have been used to create unsymmetrical dendrimers [34] and dendrimers with various core functionalizations [35]. Their functionality is readily tailored, and their uniformity, size and highly reactive surfaces are the functional keys to their application [36].

Poly(propylene imine) (PPI) dendrimers

The first poly(propylene imine) dendrimer was synthesized by Vögtle et al., according to a repetitive reactions consisting of Michael addition of an amine to acrylonitrile and then reducing the nitrile groups to primary amines [37]. The fifth-generation PPI dendrimer is the highest generation that has been synthesized, and different cores such as EDA [38] and 1,4-diaminobutane (DAB) [39] are used to synthesize these dendrimers with different generations. Scheme 3 shows the synthesis steps of PPI dendrimer with



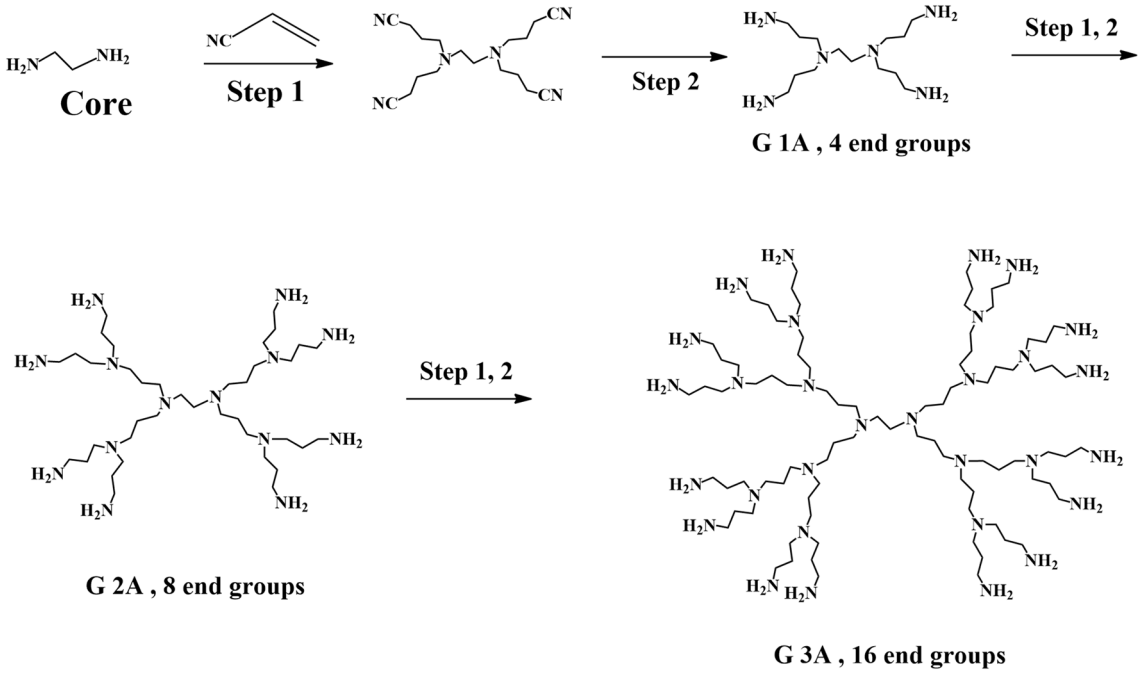
Scheme 2 Synthetic route of PAMAM dendrimer Reproduced with the permission of RSC, 2020 [4]

EDA core. Different methods have been used for reduction of nitrile groups to amines as follows:

- Using a heterogeneous hydrogenation catalyst (such as Raney nickel and cobalt), a pressure of 40 bar and a temperature of 70 °C [40];
- Using hydrazinium monoformate and Raney nickel catalyst [41];
- Using lithium aluminum hydride catalyst [42].

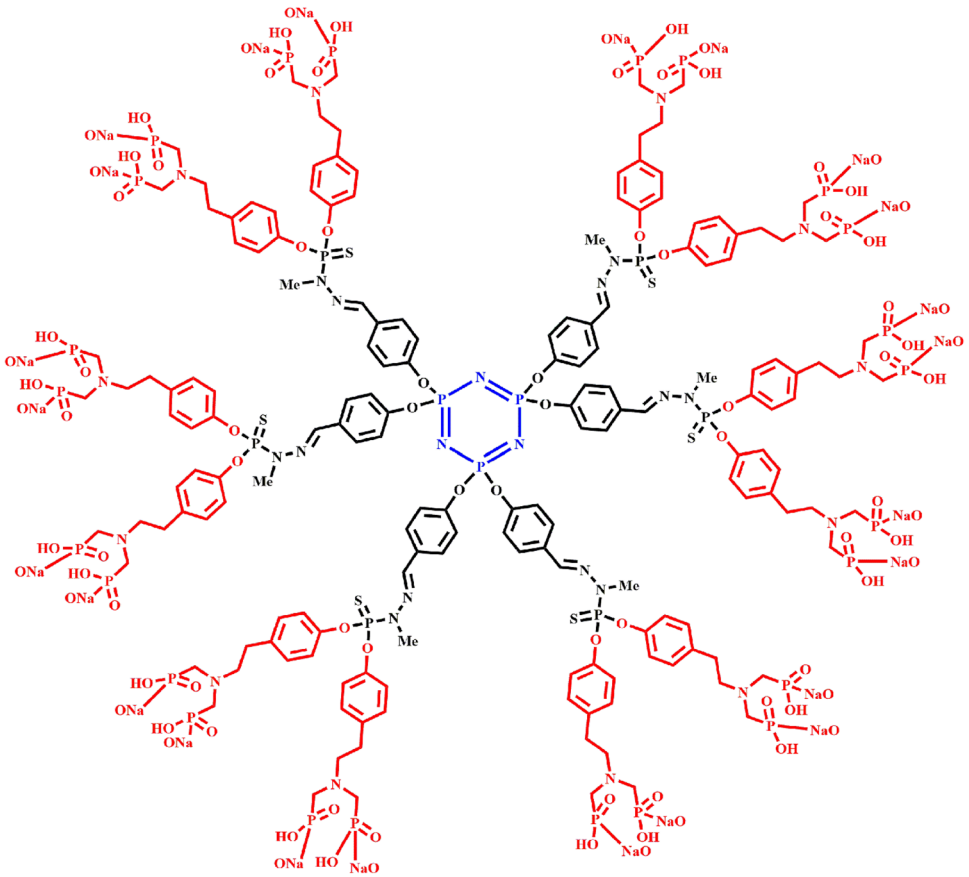
Phosphorous-based dendrimers

Rich structures of silicon- and phosphorus-based dendrimers are now available [44–48]. In recent years, because of the unprecedented attributes of phosphorous-based dendrimers such as catalyzing, materials science and medicine, they have been considered significantly [49, 50]. Several groups have described phosphate-based dendrimers using a divergent method [51, 52]. The first method was described by Regan et al. in 1990 [53]. They have synthesized a new family of dendrimers consisting of a central core and many branch points with quaternary phosphonium ion sites. In 1994, DuBois et al. [54] synthesized the first small dendrimers containing a phosphine at each branching point for electrochemical CO_2 reduction. In 1999, Kakkar and colleagues obtained larger dendrimers containing phosphine group at any point in the branching process [55]. In 1994, Majoral and colleagues presented the first neutral phosphorus dendrimers [45]. They used the following two basic steps: the reaction of hydroxybenzaldehyde and a core with P–Cl or aldehyde functions and condensation of aldehyde groups with a phosphorhydrazide [56, 57]. The attendance of aldehyde end groups or $P(S)Cl_2$ at each step can develop reactions [58–65]. However, the synthesis of these dendrimers is time-consuming. Majoral and colleagues succeeded in transforming the synthesis process into a single step using the classical method [65, 66]. They used $(S)P(OC_6H_4CHO)_3$ as a core (G0). Thus, in the first step, G1 was easily synthesized with six terminal groups of diphenylphosphino end groups using reaction between core (G0) and three equiv. of AB_2 (monomer 1). In the second step, G2 was synthesized (with 12 aldehyde terminal groups), using Staudinger reaction



Scheme 3 Synthetic route of PPI dendrimers Reproduced with the permission of Springer, 2020 [43]

Scheme 4 Structure of phosphorus-containing dendrimers [67]



between G1 and six equiv. of the azide (monomer 2). Dendrimers were formed up to the fourth generation using these two monomers in four steps. Scheme 4 shows the structure of phosphorus dendrimers.

Applications

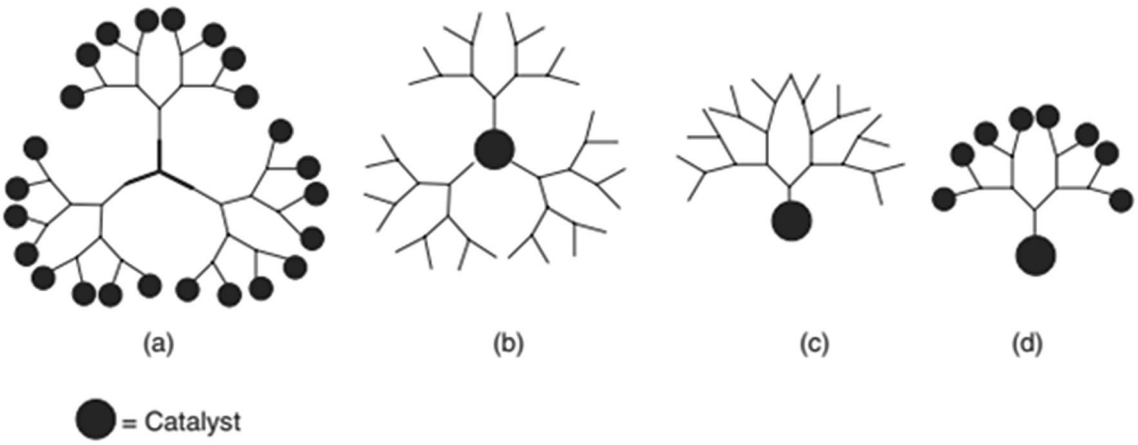
In contrast to linear polymers, which are often randomly formed, dendrimers have a specific structure that comprises a central core with branches that are located radially. In the higher generations (above 4), their structure changes to three-dimensional and quasi-spherical. The major intramolecular forces in the dendrimer are covalent bonds, but other types of interactions (such as hydrogen bonds) are known as well. They also have the ability to trap guests within the molecular space because of the ability to move their branch structure. Although many other nanostructures provide a high surface area and can be used for drug delivery [68, 69], dendrimers have good control and flexibility for this purpose. These macromolecules are very useful for carrying materials and can be organized into different dimensions. Indoor spaces such as surface end groups can be used as centers for the integration of chemical functional groups. This characteristic of dendrimers makes them useful for various applications such as catalysts [70], medicine [71], drug delivery [72] and synthesis of nanoparticles [73].

Catalysis

In recent decades, researchers have performed many different works in the field of catalysts [74–83]. Dendrimers are unique macromolecules that can be accessed using chemical compounds from a variety of building blocks. Metal complexes as catalytic groups can be located in the dendrimer core to exploit microenvironment and selectivity factors of the dendritic shell [84]. Reymond and co-workers reported the first catalytic peptide dendrimers for an ester hydrolysis reaction [85]. Dendrimers are used to prepare a particular microenvironment to simplify catalyst separation and recovery [86, 87]. In recent studies, researchers have examined peptide dendrimers such as protein mimics, antiviral and anticancer agents, vaccines and drug and gene delivery systems [88, 89]. Douat-Casassus et al. [90] synthesized different peptide dendrimers based on the Fmoc-protected 3,5-diami-nobenzoic acid as a building block for the branching unit and bearing the catalytic triad amino acids serine, histidine, and aspartate at variable positions on the dendrimer branches. As catalysis is relatively easy to tune the structure, size and location of catalytically active sites, it can be one of the most promising applications of dendrimers [91]. Host dendrimers for metal nanoparticles are catalytically active for the following reasons [92]:

- Dendrimers have a relatively uniform structure and composition;
- Nanoparticles are stabilized within the dendrimer internal cavity, which prevents their aggregation during the catalytic reaction;
- Nanoparticles inside the dendrimers are maintained by steric effects so that there is a significant portion of their inactive levels to participate in the catalytic reactions;
- To control the access of small molecules to the encapsulated (catalytic) nanoparticles, the branches of dendrimer can be used as selective gates;
- The hybrid nanocomposite solubility can be controlled with the dendrimer periphery.

Catalytic performance is measurable by sustainability, activity, selectivity, and recyclability. As shown in



Scheme 5 Various dendritic architectures: catalyst located at the periphery (**a**), internal core (**b**), focal point of a wedge (**c**) and periphery of a wedge (**d**) Reproduced with the permission of Elsevier, 2020 [95]

Scheme 5, this depends on the dendritic architecture including distinguishing periphery-, core- and focal point-functionalized dendrimers [93].

In 2000, Chechik and Crooks [94] reported Pd-encapsulated dendrimer nanoparticles as highly selective and active fluorous biphasic catalysis. They showed that these catalysts could be easily recycled and used for multiple reactions. In 2001, Crooks et al. [95] synthesized metal nanoparticles-encapsulated dendrimers and evaluated their catalytic applications. In 2006, Hoover et al. [96] used dendrimer nanoparticles (DENs) as precursors of Pt–Cu catalysts where the effect of particle composition on heterogeneous catalysts was investigated. Karakhanov et al. [97] reported thermo-responsive ruthenium catalysts based on PPI dendrimers cross-linked with poly(ethylene glycol) diglycidyl ether. The results showed good chemical and physical attributes of the synthesized catalysts such as metal loading, mean particles size, surface structure, etc. Also, dendrimers-based catalysts have many other applications including membrane reactor [98–101], biphasic systems [102], "tea bag" [103] and ionic liquids [104, 105].

Medications and in pharmaceutical

Dendrimers are used for many medications and in pharmaceutical due to the well-defined 3D structure, surface functional groups and low size besides predetermined molecular weight [106, 107]. They are combined with drugs and bioactive molecules, and their internal cavities can also be changed for combination of hydrophobic and hydrophilic drugs [108, 109]. Modified surface end groups have also been used to attach antibodies and bioactive substances and increase reactivity and solubility [110, 111]. Mechanisms of interactions between drugs and dendrimers like other polymeric structures are categorized into three main classes, namely encapsulation, electrostatic interactions and covalent conjugations [112–116].

Drug delivery systems

The central core and its internal units of dendrimers create cavities as environment for drug placement. The solubility and chemical behavior of these macromolecules can be controlled by binding the target functional groups to their surface [110]. In 1982, Maciejewski proposed the use of dendrimers as molecular containers [117]. In 2005, Patri and colleagues synthesized fifth-generation PAMAM dendrimer conjugates with folic acid and then examined the solubility of dendrimer conjugates and compared the efficacy of covalently bounded methotrexate (MTX) onto fifth-generation PAMAM dendrimer [118]. In 2012, Wang and colleagues used PPI dendrimer with varying degrees of acetylation and encapsulated drugs including sodium methotrexate and doxorubicin [119]. Acetylation of more than 80% of functional groups significantly reduced cell cytotoxicity in MCF-7 and A549 cell lines. They found that the loading capacity of drug was proportional to the degree of acetylation and increasing degree of acetylation resulted in higher loading capacity of the drug. In 2014, Kesharwani et al. [120] loaded third-, fourth- and fifth-generation PPI dendrimers with melphalan drug under identical conditions. They found that increasing generation of dendrimer led to higher drug loading due to increased internal cavities of dendrimer [120]. In 2014, Pourjavadi et al. investigated pH-responsive magnetic nanoparticles to control the release of DOX. They have grown a third-generation PAMAM dendrimer on the surface of magnetic iron oxide nanoparticles. Then, surface amines were modified with poly(ethylene glycol) dimethyl ester and then loaded the DOX onto the surface [121]. In 2017, Golshan et al. [122] functionalized fifth-generation PPI dendrimer with folic acid to target DOX delivery at different pH values. In other works, surfaces of gold nanoparticles and cellulose nanocrystals were modified with fifth-generation PPI dendrimer and release behavior of DOX was investigated at different pH values [123, 124]. In 2019, Najafi et al. synthesized gold/dendrimer hybrid nanoparticles using fifth-generation PPI dendrimer and investigated DOX release behavior and found that drug cumulative release was increased with increasing grafting density of dendrimer [23].

Brain tumor

Throughout the world, cancer has appeared as a basic cause of mortality in the world. Among the various types of cancer, brain tumor has the highest risk for life. Different types of dendrimers such as PAMAM [125, 126], PPI [127, 128] and PLL [129, 130] are used to treat and diagnose brain tumors and other cancers [131]. The greatest obstacle is often not drug potency but the physical barriers present at distinguished interfaces containing the blood vessels of the brain (blood–brain barrier, BBB), the choroid plexus (blood–cerebrospinal fluid barrier, BCSFB) and the arachnoid layer of the meninges (blood–arachnoid layer), interpreting the typical circulatory routes of delivery as ineffective [132, 133]. Many chemotherapy drugs do not reach the brain because they are substrates of the efflux transporters at the BBB. To resolve this matter, delivering chemotherapy by nanocarriers presents an attractive method [134]. Dendrimers can be easily delivering the drugs across the BBB due to their size, higher drug loading and controlled drug release [135]. Dendrimers with targeting abilities cargoe drugs to the tumor sites and penetrate to brain after systemic administration [136]. Mishra et al. [137] demonstrated that hydroxyl-terminated fourth-generation PAMAM dendrimers provided site-specific delivery of small molecular drugs across the

BBB and blood–CSF barriers. Xie et al. [138] synthesized PAMAM dendrimer composites and investigated the physiochemical properties and biological effects to achieve nasal brain transport. Teow et al. [139] showed 12-fold increased permeability of PTX compared to free drug and evaluated cytotoxicity using third-generation PAMAM dendrimers loaded with PTX. Sk et al. [140] showed that PAMAM dendrimers as drug carries increased the bioavailability of natural podophyllotoxin and estramustine conjugated with PAMAM dendrimer and increased the inhibitory activity of antimitotic agents on tubulin polymerization of glioma cell survival. Patel et al. [141] synthesized PTX-conjugated PPI dendrimer for brain delivery and also showed that the prepared conjugate had a long-term efficacy and low cytotoxicity. Somani et al. [142] checked brain delivery of plasmid DNA as medicine using third-generation PPI dendrimer anchored with transferrin (Tf). Results showed increasing uptake of plasmid DNA via Tf-conjugated PPI dendrimers.

Photodynamic therapy

Peng et al. [143] improved the photodynamic efficacy of hydrophobic porphyrin using PAMAM dendrimer–porphyrin conjugates with minimized side effects. Kojima et al. [144] investigated interactions of photosensitizers between PEG-attached PPI and PAMAM dendrimers for photodynamic therapy. Taratula et al. [145] showed that phthalocyanine-dendritic complex modified with PEG and targeting LHRH moiety had significant potential for NIR fluorescence image-guided drug delivery and photodynamic therapy. Narsireddy et al. [146] conjugated fourth-generation PAMAM dendrimer with a peptide for targeted in vivo photodynamic therapy. Lee and Kim [147] reported a hydrophilic nanoconjugate to enhance PDT efficacy by improving water solubility and intracellular uptake of Ce6.

MRI

Dendrimers are a class of compounds with great potential for use as MRI diagnostic or theranostic agents [148]. In the early 1990s, the first in vivo diagnostic imaging applications using dendrimer-based MRI contrast agents were demonstrated by Lauterbur et al. [149]. Wiener et al. introduced the first new class of dendrimer-based metal chelating as MRI contrast agent [149]. In 2001, Konda et al. [150] used folic acid-conjugated fourth-generation dendrimers as MRI contrast agent. Results showed longitudinal relaxation rate at T1 by over 100% in cells expressing the folate receptor, compared to untreated cells. Wang et al. [151] showed that a second-generation PPI dendrimer had higher relaxivity than the corresponding ammonia core PAMAM agent. Haribabu et al. used multifunctional G3 PAMAM dendrimers as T1 and T2 contrast agents for MRI [152].

X-ray contrast agent

X-ray is a useful imaging device for organs and tissues that is used in many clinical trials [153]. Guo et al. [154] modified fifth-generation PAMAM dendrimer with gold nanoparticles in different concentrations, and results demonstrated that these nanoparticles were more effective than iodine-based contrast agents for X-rays imaging. Liu et al. [155] offered the synthesis of fifth-generation PAMAM-stabilized silver nanoparticles for X-ray computed tomography (CT) imaging applications. Kojima et al. developed AuNPs-loaded PEGylated-PAMAM dendrimers for CT imaging [156–158]. Zhu et al. [159] used multifunctional AuNPs-trapped PAMAM dendrimer as a template for efficient targeting of cancer cells and X-ray attenuation.

Dendrimer as molecular probe

In 2005, Cotle et al. reported the ensemble and single-molecule (SM) dynamics of Forster resonance energy transfer in a multichromophoric rigid polyphenylenic dendrimer [160]. Kim et al. [161] synthesized biocompatible fluorescent dendritic nanoprobes containing multiple covalently linked organic dyes for fluorescence imaging.

Gene therapy

Human diseases which are transmitted to specific cells by genetic material are diagnosed and treated by gene therapy [162]. Dendrimers are used in gene delivery because of monodispersity, functional groups and multivalence structures [163, 164]. Haensler et al. [165] used dendrimers for gene therapy. Li et al. [166] modified gold nanoparticles with fifth-generation PAMAM dendrimer for a safe delivery system as controlled gene delivery for breast cancer therapy. Wang et al. synthesized aptamer-conjugated PAMAM dendrimer nanoparticles for targeted gene delivery. Results showed that dendrimer improved cellular uptake in A549 cell line and enhanced gene transfection efficiency [167]. Luong et al. used PEGylated-PAMAM dendrimers for enhancing efficacy and mitigating toxicity for effective anticancer drug and gene delivery [168]. Amreddy et al. [169] used folic acid-conjugated PAMAM dendrimer for targeted combined delivery of drug and gene to improve bioavailability and enhance therapeutic effects.

Waste water treatment

Different methods are used to treat wastewater which include dialysis [170], reverse osmosis [171], ion exchange [172], electrostatic interactions [173, 174], etc. Many adsorbents for wastewater treatment have limitations such as low absorption capacity, lack of economics of operation and

fast adsorption rates [175–177]. Due to the large number of cavities between their branches, dendrimers have high absorbance properties for wastewater treatment [178], and also because of amine end groups, they are the best option to accumulate metal ions [177]. The first report for the removal of heavy metals from water and soil using dendrimers was presented by Diallo et al. [179]. Peng et al. [180] used amphoteric PAMAM dendrimers as a flocculent in treating wastewater. In 2013, Barakat et al. [181] synthesized PAMAM-modified TiO_2 and examined the critical parameters which affect the ion removal including batch retention time, pH and metal ion concentration. Yuan et al. [182] evaluated the heavy-ion adsorption capacity of PAMAM-modified graphene oxide. Their results showed that dendrimers had a great ability to adsorb heavy ions, including Cu^{2+}, Zn^{2+}, Fe^{3+}, Pb^{2+}, Cr^{3+}. In 2014, Hayati et al. [183] studied thermodynamic properties of dye removal from colored textile wastewater using PPI dendrimer. Results indicated that dendrimer was an environmentally friendly material and suitable for removing paint from colored textile sewage at various temperatures. In 2017, Peer et al. [184] examined the absorption of Cd(II), Pb(II) and Cu(II) from aqueous solution using PAMAM-modified graphene oxide. They also studied the effects of pH, the dose of adsorbent, the contact time, Cd(II), Pb(II) and Cu(II) ions concentration, temperature of aqueous solution and thermodynamic properties (enthalpy, entropy and Gibbs free energy).

Conclusions

Unlike linear polymers, dendrimers are nano-macromolecules which branch out of a core and all the branches eventually reach a central core. In synthesis of dendrimers, their molecular size and weight can be controlled. The presence of a large number of terminal branches increases the solubility and reactivity of the dendrimers. The solubility of dendrimers is strongly influenced by the nature of the surface groups. Initiators are not used to construct dendrimers, which causes their low toxicity. Dendrimers also have a high drug delivery capacity. The unique properties of dendrimers such as controlled size, monodispersity and reactive surface groups make these molecules ideal for medical applications including biomedical, drug delivery, catalysis, etc.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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