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Dendrimer as nanocarrier for drug delivery

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ABSTRACT

Dendrimers are novel three dimensional, hyperbranched globular nanopolymeric architectures. Attractive features like nanoscopic size, narrow polydispersity index, excellent control over molecular structure, availability of multiple functional groups at the periphery and cavities in the interior distinguish them amongst the available polymers. Applications of dendrimers in a large variety of fields have been explored. Drug delivery scientists are especially enthusiastic about possible utility of dendrimers as drug delivery tool. Terminal functionalities provide a platform for conjugation of the drug and targeting moieties. In addition, these peripheral functional groups can be employed to tailor-make the properties of dendrimers, enhancing their versatility. The present review highlights the contribution of dendrimers in the field of nanotechnology with intent to aid the researchers in exploring dendrimers in the field of drug delivery.

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Contents

Abbreviations: AFM, atomic force microscopy; BHA, benzhydrylamine; BSA, bovine serum albumin; CE, capillary electrophoresis; CNTs, carbon nanotubes; CPDs, cationic phosphorus-containing dendrimers; CZE, capillary zone electrophoresis; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOX, doxorubicin; DPA, 2,4-dichlorophenoxyacetic acid; DSC, differential scanning calorimetry; DTPA, diethylene triamine pentaacetic acid; EDTA, ethylenediamine tetraacetic acid; ESI, electrospray ionization; ESI-MS, electrospray ionization-mass spectrometry; FA, folic acid; FAB-MS, fast-atom bombardment-mass spectrometry; FITC, fluorescein isothiocynate; FRET, fluorescence resonance energy transfer; FT-ICR MS, fourier transform ion cyclotron resonance mass spectrometry; FT-IR, fourier transform infrared spectroscopy; GFP, green fluorescence protein; GOx, glucose oxidase; Hb, hemoglobin; HCT, haematocrit; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HPLC, high performance liquid chromatography; HRP, horseradish peroxide; HSV, herpes simplex virus; LC, liquid crystalline; LDH, lactate dehydrogenase; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MAP, multiple antigen peptides; MPEG, methoxy polyethylene glycol; MRI, magnetic resonance imaging; MS, mass spectrometry; MTD, maximum tolerated dose; MTX, methotrexate; NMR, nuclear magnetic resonances; NSAIDs, non steroidal anti-inflammatory drugs; OS, organosilicon; PAGE, polyacrylamide gel electrophoresis; PAMAM, polyamidoamine; PAMAMOS, PAMAM-organosilicon dendrimers; PAMAM-SAHs, PAMAM succinamic acid dendrimers; PEI, polyethylenimine; PPI, poly (propylene imine); PSMA, prostate-specific membrane antigen; PTX, paclitaxel; PVP, polyvinyl-pyrrolidone; RES, reticuloendothelial system; RNase, ribonuclease; RST, repetitive synthesis technique; SANS, small-angle neutron scattering; SAXS, small-angle X-ray scattering; SEC, size exclusion chromatography; siRNA, small interfering RNA; TEER, transepithelial electrical resistance; TEM, transmission electron microscopy; TGA, thermogravimetric analysis; TLR4, Toll-like receptor 4; UPLC, ultra performance liquid chromatography; WBCs, white blood corpuscles; XRD, X-ray diffraction.

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1. History and introduction

Natural as well as synthetic polymers have always fascinated the scientists in each and every area of modern research. The synthetic polymers, particularly the biodegradable polymers have further enhanced the drug delivery applications of novel drug delivery systems. Nanobiopolymers are further refining the spectrum of pharmaceutical and biomedical applications. In this context dendrimers have emerged as one of the most promising innovative polymeric nanocarriers for different therapeutic categories of bioactives.

In the present context of architectural chemistry considerable efforts have been dedicated to development of polymer systems. The 20th century has witnessed impressive innovations in polymer synthesis and advances in the design of biodegradable polymeric macromolecules. The dendrimers are the result of these advances and innovations in the field of polymer science. Dendrimers were, for the first time, synthesized during 1970–1990 by two different groups; Buhleier et al. and Tomalia et al. In contrast to linear polymers, dendrimers developed by these two groups have precisely controlled architecture with tailor-made surface groups, which could be finely tuned [1,2].

Initially very few laboratories got interested in developing synthetic routes to these aesthetically pleasing macromolecules but in the later few years it was realized that dendritic polymers have something very special to offer. As a result, continual research efforts in this area have mushroomed and at present a growing number of publications are available, which walk around the exploration of dendrimers in the delivery of different bioactives i.e. drugs, oligonucleotides, enzymes and vaccine etc. [3–6].

The potential of dendrimers as vessels or hosts for other molecules as "dendritic box" was strikingly demonstrated by Jansen et al. [7] followed by Zimmerman et al. who investigated the fabrication as well as drug delivery propensity of benzyl ether dendrimers intervened by hydrogen bonds. The scientists had prepared a wedge-like molecule with dendritic tail and allowed six of these wedges to assemble themselves into a pie like hydrogen bonded aggregate [8]. After a long journey of break through, the credit for discovering the "Dendrimer Technology" i.e. the accurate and validated method to design a complete dendrimer goes to the pioneer chemist in the field of dendrimer i.e. Professor Donald A. Tomalia [2–10].

The expression dendrimer is derived from a Greek term *dendron* that means "tree", which is logical in view of their typical structure with a number of branching units. Dendrimers are defined as synthetic macromolecules characterized by high branching points, three dimensional globular shape, monodispersity and nanometric size range. In literature, these are also popularly described as "Cascade molecules", "Arborols", "Dendritic molecules"; or as "nanometric architectures" because of their nanoscopic size and monodispersity [9,10]. The characteristic architecture of dendrimers provides a well-defined branched structure with globular shape, which renders a large number of surface groups that can be tailored to provide a template for drug delivery [10]. Dendrimers are globular, nano-sized (1–100 nm) macromolecules with a particular architecture constituted of three distinct domains: (i) a core at the center of dendrimer consisting of an atom or a molecule having at least two identical chemical functions; (ii) branches, emanating from the core, constituted of repeat units having at least one branch junction, whose repetition is organized in a geometrical progression that results in a series of radially concentric layers called "generations"; and (iii) many terminal functional groups, generally located at the surface of dendritic architecture. These surface groups are vital in determining the properties of dendritic macromolecules [11] (Fig. 1).

2. Properties of dendrimers

Dendritic architecture holds immense potential over the other carrier systems, particularly in the field of drug delivery, because of their unique properties. As compared to traditional linear polymers, dendrimers exhibit significantly improved physical and chemical properties. Salient properties of dendrimers are discussed below.

2.1. Monodispersity

Dendrimers are the class of dendritic polymers that can be constructed with a well-defined molecular structure, i.e. monodisperse unlike linear polymers. Monodispersity offers researchers the possibility to work with a tool for well-defined and reproducible scalable size [12]. Monodispersity of dendrimer has been confirmed widely by mass spectroscopy, size exclusion chromatography (SEC), gel electrophoresis and transmission electron microscopy (TEM). Due to the purification at each step of synthesis the convergent methods generally produce the most nearly isomolecular dendrimers [11–14]. Mass spectroscopy data have well established that PAMAM dendrimers produced by the divergent method are extremely monodisperse for earlier generation (1.0–5.0 G). Factors such as dendrimer bridging and incomplete removal of ethylenediamine at each of the generation sequences may affect the degree of monodispersity [2]. This latter factor, at any point in dendrimer growth, will function as an initiator core to produce 0.5 G and subsequent generation dendrimers that leads to polydispersity [15].

2.2. Nano-size and shape

Dendrimers with uniform and well-defined size and shape are of prominent interest in biomedical applications because of their ability to cross cell membranes as well as reduce the risk of premature clearance from the body. The high level of control over the dendritic architecture makes them an ideal carrier. The size of dendrimers increases systematically, as does the generation number, ranging from several to tens of nanometers in diameter. Dendrimers are similar in size to a number of biological structures, for example, 5.0 G PAMAM dendrimers is approximately the same size and shape as hemoglobin (Hb) (5.5 nm diameter) [16]. Several classes of dendrimers have been synthesized with a variety of core materials, branching units and surface modifications. Dendrimer size will also be relevant to the three dimensional shape; lower generation dendrimers

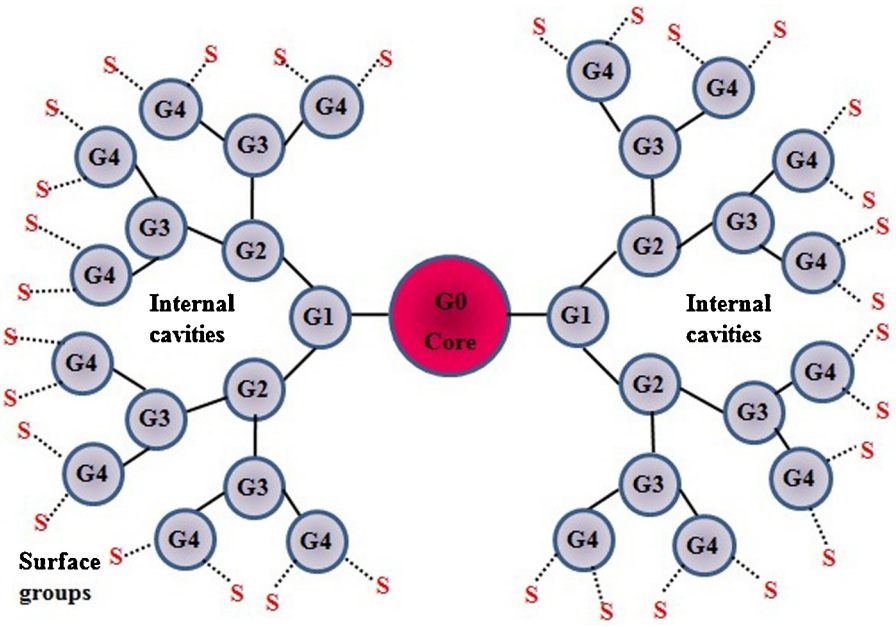

Fig. 1. Schematic representation of general structure of dendrimer.

tend to be open and amorphous structures whereas higher generations can adopt a spherical conformation, capable of incorporating drug molecules. X-ray analysis on dendrimer aggregates suggested that the molecular shape of the lower to higher generations becomes increasingly globular (i.e. more spherical compared to linear shape), in order to spread out the larger molecular structure with a minimal repulsion between the segments [17].

2.3. Biocompatibility

Regardless of their toxicity dendrimers have been considered as 'smart' carrier owing to their ability as intracellular drug delivery vehicle, to cross biological barriers, to circulate in the body during time needed to exert a clinical effect, and to target specific structures. Toxicity of dendrimers is ascribed mainly to the end group present on its periphery. Generally amine-terminated PAMAM and PPI dendrimers display concentration-dependent toxicity and hemolysis [18–20] whereas neutral or anionic groups terminated dendrimers have shown comparatively less toxicity and hemolysis [18,21–24]. Fortunately the toxicity of cationic dendrimers can be overcome by partial or complete modification of their periphery with negatively charged or neutral groups [18,20,25]. Although both PAMAM and PPI dendrimers have terminal amino groups yet they display different pattern of toxicity. In case of cationic PAMAM dendrimers, toxicity increases with each generation but unpredictably cationic PPI dendrimers do not follow this pattern of toxicity [18,19]. The cytotoxicity behavior of cationic dendrimers is widely explained by the favored interactions between negatively charged cell membranes and the positively charged dendrimers surface, enabling these dendrimers to adhere to and damage the cell membrane, causing cell lysis. Whereas masking of cationic end groups or conversion of end groups of dendrimers to neutral or anionic groups have resulted in dendrimers with decreased toxicity or even non-toxic dendrimers in both *in vitro* and *in vivo* studies as observed in case of neutral dendrimers like polyester, polyether and surface engineered dendrimers, for example glycosylated, PEGylated dendrimers etc. [21,26,27].

2.4. Periphery charge

Dendrimers consist of three structural units i.e. core, branching units and a number of terminal end groups. End groups may possess positive, negative or neutral charges, which are vital in the exploration of dendrimers as drug delivery vehicles. This polyvalency can be exploited to play an important role in the application of dendrimers as gene carrier because cationic dendrimers like poly-L-lysine, PPI and PAMAM, etc. can form complexes with negatively charged DNA. Also the positive charges of dendrimers facilitate its interaction with negatively charged biological membranes leading to applicability of dendrimers for intracellular drug delivery. Challenging these advantages, the polyvalency of dendrimers also leads to the toxicities including cytotoxicity, hemolysis etc. [18,28]. Fortunately these toxicities can be overcome by surface modification (engineering) of dendrimers with different agents like carbohydrates, PEG, acetate etc. Thus polyvalency has important implication on the properties of dendrimers and provides a potential arena for scientists working in the field of dendrimers-mediated drug delivery [25,29], the focal theme of this review.

Sadekar and Ghandehari, reviewed the role of PAMAM dendrimers in oral delivery with effect of surface charge and generation on the toxicity as well as transepithelial transport and cellular uptake. In studies with Caco-2 cell

lines that these dendrimers were found to be transported by both routes; paracellular via opening of tight junction as well as transcellular mediated by endocytosis. The surface engineering of PAMAM dendrimers resulted in both reduced toxicity as well as enhanced transepithelial transport. In addition to this, authors also performed the critical *in vivo* evaluation of PAMAM dendrimers-based conjugates to elucidate their efficacy, toxicity and stability [30].

2.5. Dendrimer–membrane interactions

Interaction of higher generation dendrimers having positively charged surface groups with negatively charged biological membrane results in formation of nanoscale holes and cell lysis. Two most widely used models to understand this mechanism include biological membranes as well as living cell membranes [31–34]. The interaction of cationic phosphorus-containing dendrimers (CPDs) (3.0 G and 4.0 G) with model lipid membranes has been investigated. Model lipid bilayers consisting of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and dispersed in aqueous HEPES buffer solution (10 mM, pH 7.4) were used and then interactions were studied with differential scanning calorimetry (DSC) and zeta-potential techniques. Result of calorimetric analysis displayed generation-dependent interaction. Presence of dendrimers attributed significant changes in the main transition enthalpy and phase transition temperature values. Rate of alteration of thermotropic behavior was found to be concentration-dependent. The fluidity rate of the lipid–dendrimer complexes was proportional to the dendrimer/lipid molar ratios [35].

The effect of PAMAM dendrimers on paracellular permeability, integrity, and viability of Caco-2 cell monolayers was investigated by measuring the mannitol permeability, transepithelial electrical resistance (TEER) and lactate dehydrogenase (LDH) enzyme leakage, respectively. The concentration of dendrimers, incubation time and generation increase were found to be directly proportional to LDH leakage [36]. Mecke et al. suggested that in the fluid phase of a membrane, cationic PAMAM dendrimers leads to hole formation however the existence of a gel phase in the plasma membrane is unaffected by the presence of these dendrimers [37].

Tiriveedhi et al. studied the interaction of 1.0 G and 4.0 G PAMAM dendrimers with model lipid membranes using the fluorescence spectroscopy and surface tensiometry techniques. As examined with fluorescence anisotropy, electrostatic interaction was found to mediate the binding between dendrimers and membrane owing to positive surface charge and negative charge of dendrimers and membranes, respectively. Finally authors concluded that the PAMAM dendrimers of low generation penetrate into lipid monolayer at low surface pressure (<30 mN/m) and high fluidity as elucidated with surface tensiometry. From this study it was inferred that lower generation PAMAM dendrimers interact electrostatistically with membrane by inducing aggregation of lipid vesicles without affecting integrity of membrane significantly. Further, this interaction was affected by two parameters; membrane fluidity and surface pressure with preferential interaction at subphysiologic surface pressure in liquid crystalline state, particularly evident in case of rapidly dividing cells [38]. Lower generation cationic dendrimers interact electrostatically with biological membrane without affecting its integrity but the higher generation dendrimers with large density of cationic charges interact with membrane to induce formation of nanoholes and may lead to cell death followed by toxicity.

2.6. End groups and toxicity

Dendrimers being nanometric size can non-specifically, interact with a variety of cells and cellular components menifesting toxic consequences. It has been reported that in case of dendrimers the cell toxicity is associated with the number of end groups and surface charges. Cationic dendrimers like PAMAM, PPI and poly-L-lysine have shown toxicity in a dose-dependent manner however negatively charged dendrimers such as sulfonated, carboxylated, phosphonated; or neutral dendrimers, such as dendrimers with poly(ethylene oxide), acetyl, carboxyl, mannose, galactose end groups; revealed less toxicity compared to positively charged dendrimers. In light of these reports, modification of surface groups of cationic dendrimers with neutral molecules is preferred to possibly prevent toxicity of dendrimers [28,35–38] (Fig. 2). As discussed in the previous section dendrimer having positively charged endgroups may interact with negatively charged membrane and may increase the permeability that facilitates the intracellular delivery of bioactives. But in case of higher generation dendrimers, dendrimer–membrane interaction may result in disruption of membrane integrity followed by leakage of important intracellular components, which finally leads to cell death and toxicity. This toxicity is attributed to high charge density inherently associated with larger generations of positively charged dendrimers.

2.7. Pharmacokinetics

Pharmacokinetics of macromolecules depends primarily upon the anatomical and physiological characteristics, the physicochemical properties of macromolecules and their interactions with biological components [39]. In case of intravenous administration, macromolecules are instantly introduced into the blood circulation with restricted diffusion to the extravascular space. Subsequent elimination of these circulating macromolecules occurs followed by distribution to particular organs for disposition, which reflects the plasma clearance by these organs or tissues. Factors that play a major role in the specific tissue-macromolecules uptake are capillary permeability, organ blood flow and nature of the macromolecules.

In case of dendrimers, parenteral administration has been utilized mostly suggesting the potential for dendrimer absorption across various epithelial barriers, including the intestine and the skin [40]. Ideally, dendrimers are developed to deliver drugs to target tissues to obtain adequate therapeutic efficacy, avoiding toxic effects on the healthy bystander cells, which are associated

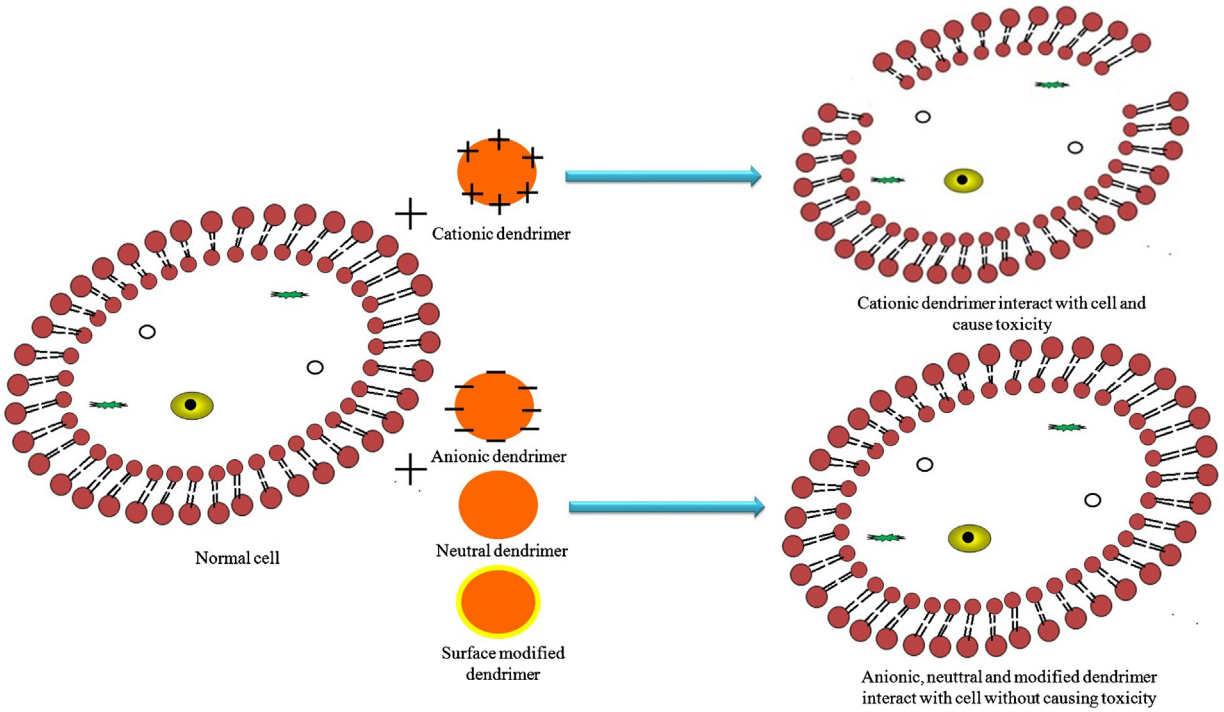

Fig. 2. Biological interaction of cell with cationic, anionic, neutral and surface modified dendrimer.

with the potential organ-specific toxicity of the free drugs. Surface decorated dendrimers are reported to exhibit improved pharmacokinetic profiles than plain PPI dendrimers [21,41].

Pharmacokinetics of intravenously administered tritiated poly-L-lysine dendrimers has been reported. Very rapid initial clearance and unpredictably high initial volumes of distribution were observed in case of amine-terminated 3.0 G and 4.0 G dendrimers [42]. In another study, effect of surface charge on excretion profile of non-biodegradable 5.0 G PAMAM dendrimers was compared. As compared to cationic dendrimer, about twice as much of the uncharged dendrimer was excreted via the urine and feces over 7 days; suggesting enhanced cellular uptake of the cationic dendrimers [43].

Rapid vascular binding of dendrimers with surface amino groups may reduce on conjugation with anionic capping groups, which conceals the surface amine group. Subsequently the systemic disposition is governed by the renal and reticuloendothelial system (RES) clearance. It was found to be dependent on the nature of the anionic group [18,44]. Nigavekar et al. evaluated the biodistribution of ^{3}H-labeled 5.0 G PAMAM dendrimers (positively charged) and acetylated dendrimers in B16 melanoma and DU145 prostate tumor models and concluded greater tissue deposition in case of positively charged PAMAM [43]. Although, enough reports are available on the pharmacokinetics of dendrimers yet systematic investigation on the *in vivo* fate of dendrimers is urgently needed to this myriad drug delivery system to certify its clinical utility.

3. Macromolecular architecture of dendrimers

The dendritic architecture has been stated as the fourth major class in the family of macromolecular polymeric architecture after linear, cross linked and branched molecular architecture. According to Tomalia [45], this fourth class of macromolecular architecture could be further subdivided into four dendritic or cascade molecule subclasses namely random hyperbranched polymer, dendrigraft polymers, dendrons, and dendrimers (Fig. 3).

During the production of dendrimer each step is controlled throughout the chemical synthesis that results in monodisperse, macromolecular, globular polymeric architecture. This globular macromolecular architecture of dendrimers has a large number of surface groups, which can be easily tailored to achieve various objects [46] (Fig. 4). Dendritic architecture is synthesized either by divergent or convergent approaches [47]. In divergent method dendrimer is grown away from a central focal point i.e. core extending radially to the periphery; whereas in convergent method, synthesis starts from the surface and proceeds towards the interior prior to the attachment of pre-synthesized dendrons to the core. Since dendrimers are synthesized by step-by-step growth method, the branching elements are depicted by generation number. The core molecule is demonstrated by generation 0 (G_0) whereas successive addition of branching units leads to higher generations G1, G2, G3. . . . . and so on. Fig. 5 portrays the different generations of ethylene diamine cored poly (propylene imine) (PPI) dendrimers. Both of these methods result in exponential increase in the

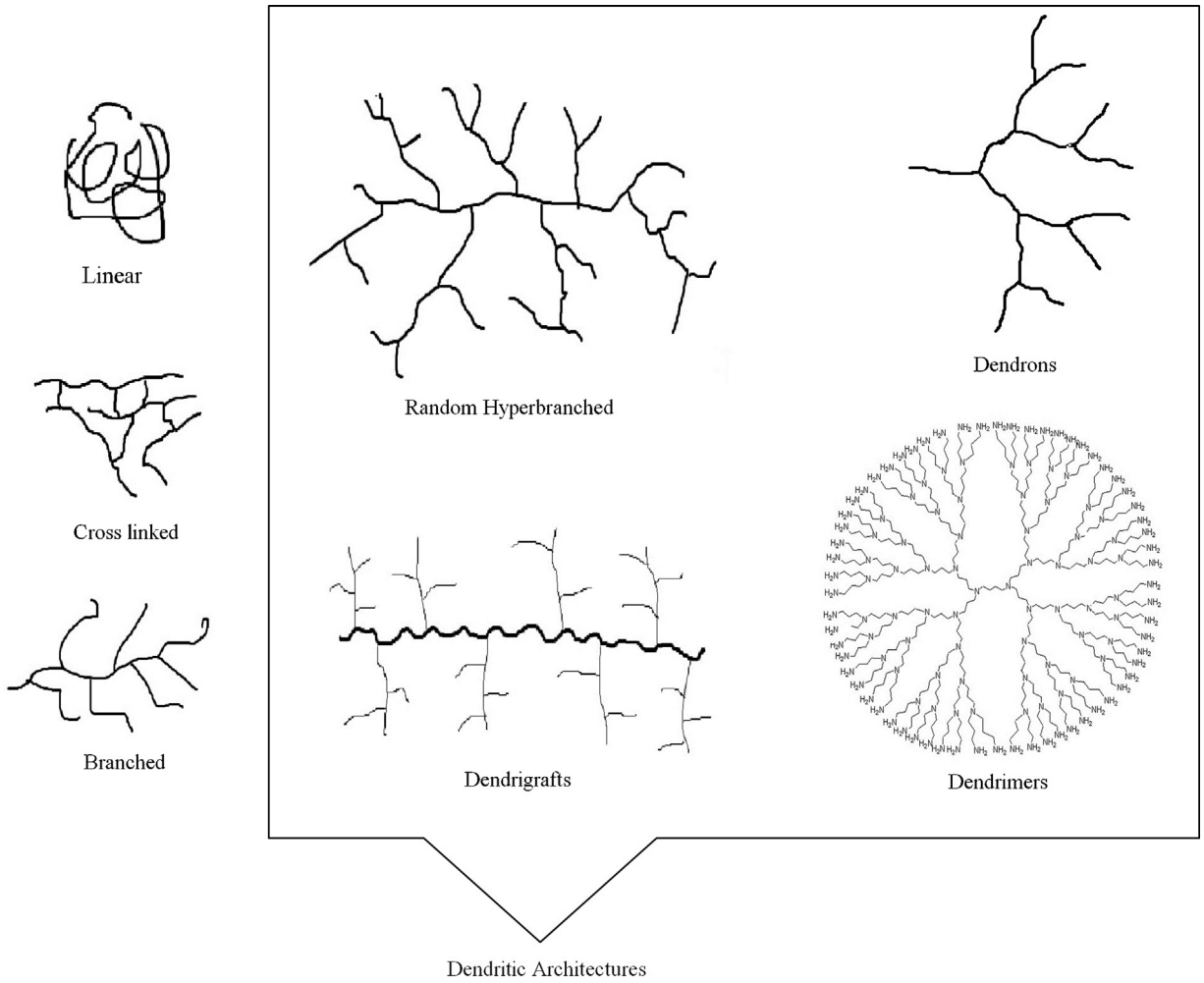

Fig. 3. Schematic representation of various classes of polymeric architecture.

number of functional groups with each successive generation. This exponential increase in functional groups leads to steric crowding, which finally results in geometrical changes. Initially diameter of dendrimer increases linearly however in later stage dendritic polymer adopts more globular shape with increase in dendrimer generation due to steric hindrance [13,45,47,48]. These unique structural features ascribe the dendrimers ideal architectural properties including monodispersity, hyperbranched polymeric architecture, definite nanometric size, shape, molecular weight and stability. In this way, dendrimer has widely been explored as a new platform for delivery of bioactives owing to unique biological properties such as high drug pay load, lipid bilayer interactions, targeting potential, blood plasma retention time, filtration, intracellular internalization, biodistribution, transfection, good colloidal and biological stability (Fig. 6). A variety of dendritic molecules have been explored thoroughly for drug delivery including polyamidoamine (PAMAM), PPI, poly-L-lysine, triazine, melamine, PEG and carbohydrate-based citric acid, poly(glycerol-co-succinic acid), poly(glycerol), and poly[2,2-bis(hydroxymethyl)propionic acid] dendrimers

Table 1
Theoretical details of different generation of PAMAM and PPI dendrimers.

Dendrimer type	PAMAM			PPI		
Generation	Surface groups	Molecular formula	Molecular weight	Surface groups	Molecular formula	Molecular weight
0	4	$C_{22}H_{48}N_{10}O_4$	517	4	$C_{14}H_{36}N_6$	288
1	8	$C_{62}H_{128}N_{26}O_{12}$	1430	8	$C_{38}H_{92}N_{14}$	746
2	16	$C_{142}H_{288}N_{58}O_{88}$	3256	16	$C_{86}H_{204}N_{30}$	1658
3	32	$C_{302}H_{608}N_{122}O_{60}$	6909	32	$C_{182}H_{428}N_{62}$	3486
4	64	$C_{622}H_{1248}N_{250}O_{124}$	14,215	64	$C_{374}H_{876}N_{126}$	7140
5	128	$C_{1282}H_{2528}N_{508}O_{252}$	28,826	128	$C_{758}H_{1772}N_{254}$	14,436

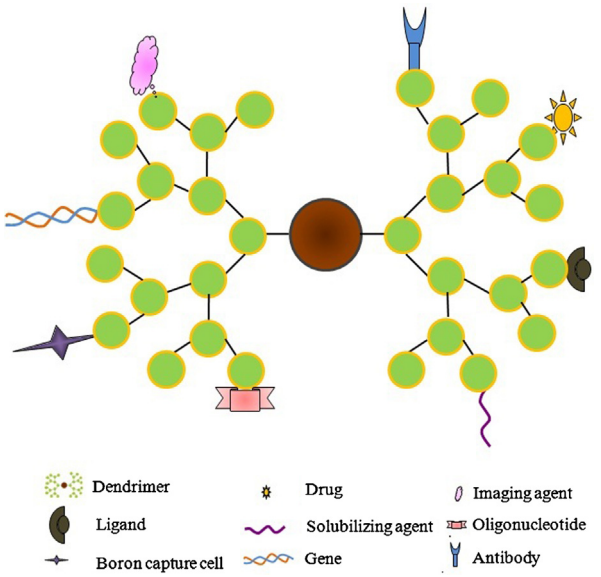

Fig. 4. Dendrimer as platform for multiple ligands as a tool for various applications.

[46,49,50] (Fig. 7). For sake of clarity theoretical details of molecular weight, molecular formula and surface groups of two most explored dendrimers, PPI and PAMAM dendrimers are summarized in the Table 1.

4. Synthesis of dendrimers

Dendrimers are symmetric, highly branched polymers with a compact spherical structure (diameter ranging from 1.1 nm for 1.0 G PAMAM to 9 nm for 8.0 G PAMAM dendrimer) [51]. They are normally synthesized from a central polyfunctional core by repetitive addition of monomers. The core is characterized by a number of functional groups. Addition of monomers to each functional group results into next dendrimer generation as well as expression of end groups for further reaction [52,53]. Size of dendrimer increases as the generation number increases; a stage will soon reach when dendrimer attains its maximum size and becomes tightly packed looking like a ball. Divergent and convergent methods are most frequently used for dendrimer synthesis [48]. In addition other approaches like 'hypercores' and 'branched monomers' growth, 'double

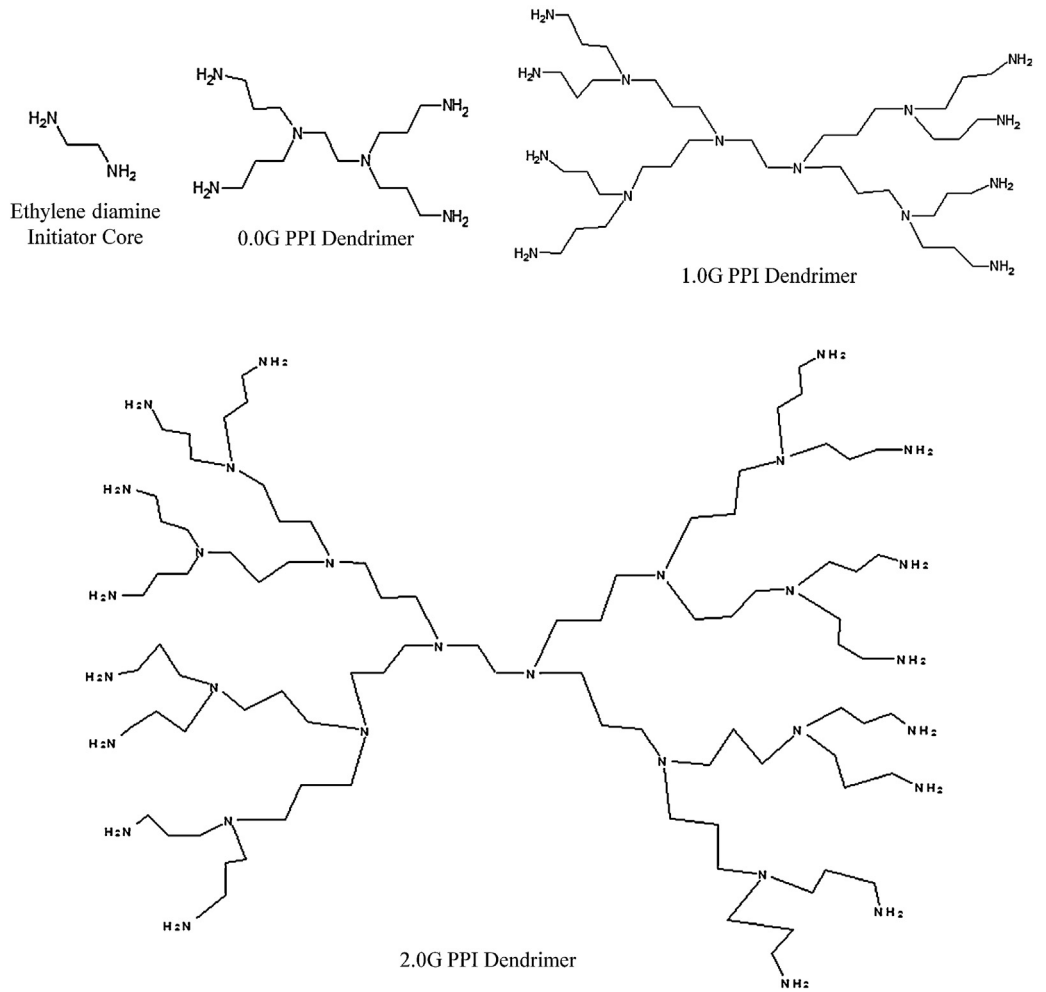

Fig. 5. Depiction of various generations of PPI dendrimers.

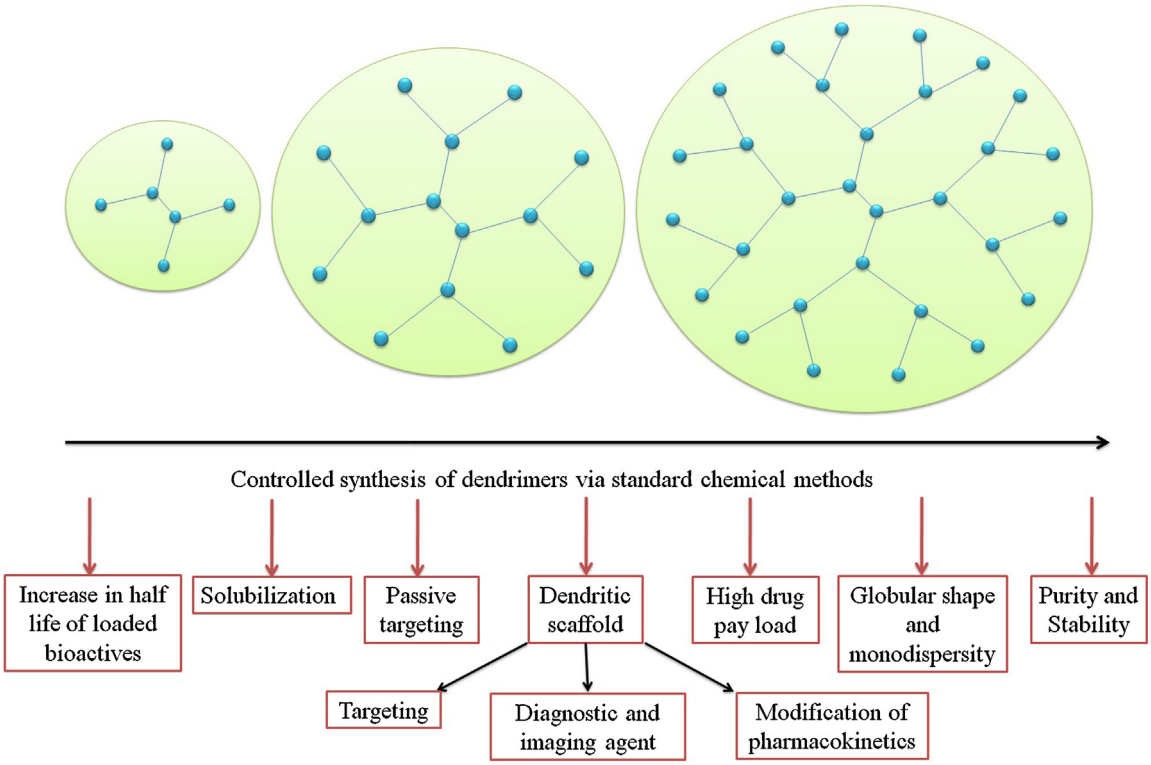

Fig. 6. Key features derived from well-defined structure and controlled synthesis of dendrimers.

exponential' growth, 'lego' chemistry and 'click' chemistry are also used (Fig. 8).

4.1. Divergent approach

Divergent approach comprises of two steps, first is the activation of functional surface groups, and second is the addition of branching monomer units [47]. In this approach, the core is reacted with two or more moles of reagent containing at least two protecting/branching sites, followed by removal of the protecting groups. This will lead to the formation of first generation dendrimer. This process is repeated several times until the dendrimer of the desired size is formed. PAMAM starburst dendrimers are prepared by this method. As compared to others method, divergent approach has some over riding advantages such as ability to modify the surface of dendrimer molecules by changing the end groups at the outermost layer. Another advantage is that the overall chemical and physical properties of dendrimer can be configured to specific need [54–56].

4.2. Convergent approach

This is an alternative method of dendrimer synthesis firstly proposed by Hawker and Frechet in 1990. Only one kind of functional group on the outermost generation is the main constraint of divergent growth method. Convergent growth would overcome such a weakness. Convergent method involves two stages, firstly a reiterative coupling of protected/deprotected branch to produce a focal point functionalized dendron; and secondly, divergent core anchoring step to produce various multidendron dendrimers. Precise control over molecular weight and production of dendrimers having functionalities in precise positions and number are some outstanding dividends of this method [13].

However difficulty to synthesize the dendrimer in large quantities, because of repeated reaction occurring during convergent approach that necessitates the protection of active site, is a significant limitation of these methods. Presently dendrimers are commercially manufactured by companies like Dentritech (Midland, US), Dutch State Mines (DSM), Netherlands, Dow Chemicals (Michigan, US), Aldrich Chemical Company (Milwaukee, WI) and Weihai CY Dendrimer Technology (China).

4.3. Other approaches

4.3.1. Hypercores and branched monomers

This method involves the pre-assembly of oligomeric species to hasten up the rate of dendrimer synthesis. In this method oligomeric species are linked together to yield dendrimers in fewer steps and/or higher yields. Essentially a hypercore having multiple attaching groups is grown from core molecule and the surface units are linked to branched monomer with focal point activation leading to synthesis of blocks, which are then attached to hypercore to generate a higher generation dendrimers [57].

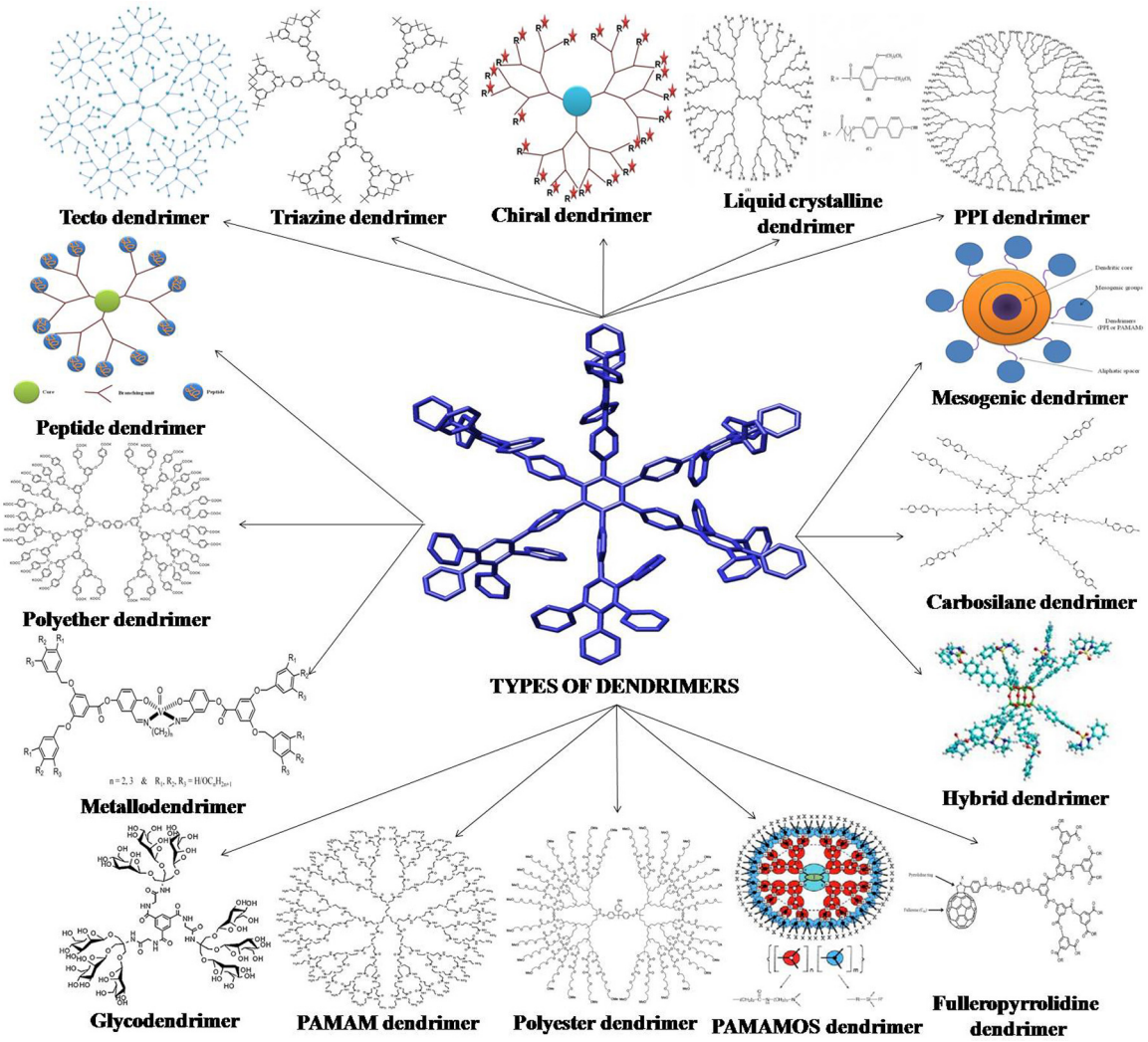

Fig. 7. Different types of dendrimers.

4.3.2. Double exponential

This approach allows the preparation of monomers for both divergent and convergent growth from a single starting material, which is similar to a rapid growth technique for linear polymer. The resultant two products are then reacted to give an orthogonally protected trimer, which can be used to repeat the growth again. Advantage of double exponential growth approach is rapid synthesis and applicability to either divergent or convergent method.

4.3.3. Lego chemistry

In order to simplify the synthetic procedure for dendrimers, in terms of cost as well as duration of synthesis, various approaches have been explored by scientists; Lego chemistry is one of the outcomes of these explorations. Lego chemistry is based on the application of highly functionalized cores and branched monomers and has been utilized in the synthesis of phosphorus dendrimers. The basic synthetic scheme has undergone several modifications and has resulted in a refined scheme wherein a single step can amplify the number of terminal surface groups from 48 to 250. Apart from higher growth in the number of terminal surface groups in few reactions, this method also encompasses the advantage of utilizing minimum volume of solvent, allowing simplified purification procedure with eco-friendly by-products like water and nitrogen [58].

4.3.4. Click chemistry

Another approach for fast and reliable synthesis of dendrimers is based on click chemistry where in small units are joined together. High chemical yield with innocuous by-product is the main characteristic of click chemistry reaction. Use of simple reaction conditions, easily available reagents, and benign solvent are the additional profits of click chemistry. Following the click chemistry strategy dendrimers with various surface groups can be obtained in high purity and excellent yield. 2.0 G and 3.0 G triazole dendrimers were synthesized using Cu (I)-catalyzed click chemisty reactions and obtained dendrimers were isolated

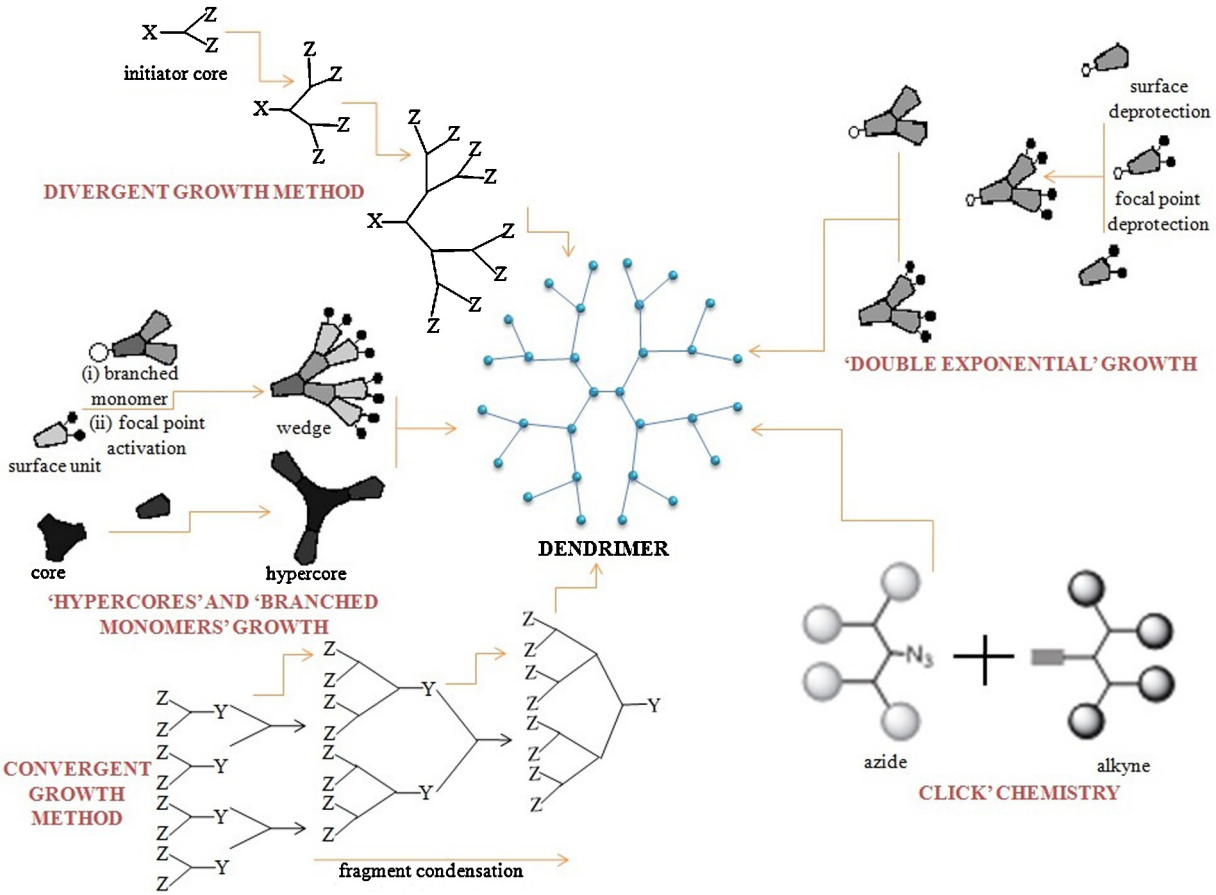

Fig. 8. Methods of dendrimers synthesis.

as pure, solid sample with only sodium chloride as the major by-product using chromatographic procedure [58].

5. Types of dendrimers

A rapid development of dendritic novel carrier has been possible due to recent advances in synthetic chemistry and characterization techniques. Also a range of dendritic scaffolds has become available with defined nanoscopic size and ample numbers of functional end groups [59]. Some of the dendrimers having different functionalities along with their applications are described in Fig. 7.

5.1. PAMAM dendrimer

The first dendritic structures that have been exhaustively investigated and have received widespread attention were Tomalia's PAMAM dendrimer. PAMAM dendrimers are synthesized by the divergent method starting from ammonia or ethylenediamine initiator core reagents. Products up to generation 10 (a molecular weight of over 930,000 g/mol) have been obtained. The polydispersity index of 5.0–10.0 G PAMAM dendrimers is less than 1.08, which means that the particle size distribution is very uniform for each generation [60]. Due to the presence of positive charge on the surface, PAMAM dendrimers have the ability for condensation of DNA followed by transfection [61].

5.2. PPI dendrimer

PPI dendrimers are amine-terminated hyperbranched macromolecules, which are mainly synthesized by divergent method [62]. PPI dendrimer contains two types of nitrogen atoms; nitrogen of primary amine and nitrogen of tertiary amine. As compared to tertiary nitrogen atoms, which are more acidic having a pK_a around 6–9, primary nitrogen atoms are more basic having a pK_a around 10. PPI dendrimers are synthesized by divergent approach, in a sequence of repetition of double Micheal addition of acrylonitrile to primary amines followed by heterogeneously catalyzed hydrogenation of nitriles. This repeated reaction results in a doubling of the number of primary amines. During the synthesis of PPI dendrimer 1,4-diaminobutane is utilized as dendrimer core. A variety of molecules with primary or secondary amine groups can also be used as core in dendrimer synthesis [63]. These PPI dendrimers are commercially available from Dutch State Mines (DSM), Netherlands and Aldrich Chemical Company (Milwaukee, WI).

5.3. Liquid crystalline (LC) dendrimers

LC dendrimers consist of mesogenic LC monomers e.g. mesogen functionalized carbosilane dendrimers. Thermotropic LC phases or mesophases are usually formed by rod-like (calamitic) or disk-like (discotic) molecules [59]. Dendrimers with AB mesogens in the branches were first reported by Percec et al. in 1995 [64]. Frey and coworkers also attached several mesogenic units to carbosilane dendrimers, such as cyanobiphenyl [65] and cholesteryl [66]. In a reported study, mesogenic 3,4-bis-(decyloxy)benzoyl groups functionalized PPI dendrimers of different generations (1.0–5.0 G) were investigated for mesogenic activity. It was found that apart from fifth generation dendrimer, all other lower generations' dendrimers displayed a hexagonal columnar mesophase in which the dendrimers had a cylindrical conformation [67,68]. The lack of mesomorphism for the fifth generation dendrimer was due to its inability to reorganize into a cylindrical shape. In 2001, Boiko et al. reported the debut synthesis of photosensitive LC dendrimer with terminal cinnamoyl groups [69]. These LC dendrimers are being investigated by scientists for biomedical applications. Recently Pedziwiatr-Werbicka and coworkers suggested that amino terminated carbosilane dendrimers have potential to deliver short chain siRNA and anti-HIV oligodeoxynucleotide to HIV-infected blood cells. Although these dendrimers had limited application in the delivery of long-chain double stranded nucleic acids, yet the dendriplexes of carbosilane dendrimers and anti-HIV nucleic acid were stable and less cytotoxic to blood cells than the plain dendrimers suggesting their utility in the delivery of bioactives [70].

5.4. Core shell (tecto) dendrimers

Core–shell or tecto-dendrimers represent a polymeric architecture with highly ordered structure obtained as a result of controlled covalent attachment of dendrimer building blocks [71,72]. Tecto-dendrimers are composed of a core dendrimer, which may or may not contain the therapeutic agent, surrounded by dendrimers. Synthesis of tecto-dendrimers has been reported with fluorescein in the core reagent for detection and folate as the targeting moiety [59,73]. Such conjugates solved the solubility problems encountered in previous studies with aromatic fluorescein isothiocynate (FITC) moieties on dendrimeric surfaces. This conjugate was found to be overwhelmingly superior to those dendrimeric conjugates containing both FITC and folic acid attached to the surface [74]. In contrast to simple dendrimers, synthesis procedure for tecto-dendrimers is complaratively simple and hence expected to inflate the application of dendrimers. Schilreff et al. investigated the cytotoxicity of tecto-dendrimers to point out their application in biomedical field. In this study tecto-dendrimers having amine-terminated 5.0 G PAMAM dendrimers as core, surrounded by shell composed of 2.5 G PAMAM dendrimers with surface carboxyl groups, were investigate for cytotoxicity towards SK-Mel-28 human melanoma cells. These tecto-dendrimers were found to inhibit growth of melanoma cells at a concentration which is safe to healthy keratinocytes epithelial cells [71]. Thus tecto-dendrimers could be explored for application in the field of nanomedicine including drug delivery.

5.5. Chiral dendrimers

Dendrimer based upon the construction of constitutionally different but chemically similar branches to chiral core is referred to as chiral dendrimers. Chiral, non-racemic dendrimer with well-defined stereochemistry is particularly interesting subclass with potential applications in asymmetric catalysis and chiral molecular recognition [75]. Ghorai et al. described the first molecules of anthracene capped chiral dendrimers derived from a 1,3,5-trisubstituted aromatic core and carbohydrate units in the interior and periphery. These are claimed to be suitable for anchoring other useful functionalities aimed at applications as drug delivery system and light harvesting materials [76]. Evidence supporting the above claim is keenly awaited, particularly in the field of drug delivery application of chiral dendrimers.

5.6. Peptide dendrimers

Peptide dendrimers are radically branched macromolecules that contain a peptidyl branching core and/or peripheral peptide chains [77], and can be divided into three categories. First category having peptides only as surface functionalities is referred to as "grafted" peptide dendrimers, the second category composed entirely of amino acids is known as peptide dendrimers, while the third one utilizes amino acids in the branching core and surface functional groups but having non-peptide branching units. Divergent and convergent methods are frequently used for the synthesis of peptide dendrimers, and the availability of solid-phase combinatorial methods facilitates large libraries of peptide dendrimers to be produced and screened for desired properties. Peptide dendrimers have been used in industry as surfactants, and in biomedical field as multiple antigen peptides (MAP) [77], protein mimics [78] and vehicles for drug and gene delivery [77,78]. Additionally, Darbre and Reymond have utilized peptide dendrimers as esterase catalysts [79].

5.7. Glycodendrimers

Dendrimers that encompass sugar moieties such as glucose, mannose, galactose [80] and/or disaccharide [81] into their structure are referred to as glycodendrimers. The vast majority of glycodendrimers have saccharide residues on their outer surface, but glycodendrimers containing a sugar unit as the central core, from which all branches emanate, have also been described. Generally, glycodendrimers can be divided into three categories (i) carbohydrate-centered, (ii) carbohydrate-based, and (iii) carbohydrate-coated dendrimers [59,82]. One anticipated application of these dendrimers is site-specific drug delivery to the lectin-rich organs. These dendrimers were anticipiated to display better association with lectins anchored systems as compared to mono-carbohydrate anchored systems [83,84].

5.8. Hybrid dendrimers

Hybrid dendrimers are combination of dendritic and linear polymers in hybrid block or graft copolymer forms. The spherical shape and a large number of surface functional groups of dendrimers made the formation of dendritic hybrids possible. The small dendrimer segment coupled to multiple reactive chain ends provides an opportunity to use them as surface active agents, compatibilizers or adhesives, or hybrid dendritic linear polymers [85]. The dendritic hybrids obtained from various polymers with dendrimers generated the compact, rigid, uniformly shaped globular dendritic hybrids, which have been explored for various aspects in the field of drug delivery [85,86].

5.9. PAMAM-organosilicon (PAMAMOS) dendrimers

Inverted unimolecular micelles that consist of hydrophilic, nucleophilic PAMAM interiors and hydrophobic organosilicon (OS) exteriors are known as radially layered PAMAMOS dendrimers (PAMAMOS). PAMAMOS dendrimers offer unique potential for novel application in electronics, chemical catalysis, nano-lithography and photonics etc., due to its unique properties such as constancy of structure and ability to form complex and encapsulate various guest species with nanoscopic topological precision [87].

Continuous urge for optimum therapeutic delivery system for various medicaments used in treatment of infectious and non-infectious diseases leads to development and investigation of various types of dendrimers like polyether dendrimers, polyester dendrimers, triazine dendrimers, melamine dendrimers, citric acid dendrimers etc. using different core and branching units. The arena of dendrimers is everexpanding and applications of this versatile carrier system in the drug delivery are inflating day by day.

6. Characterization of dendrimer

The well-defined nanometric architecture of dendrimers is the result of controlled synthesis of these moieties at each step by chemical reaction. Characterization of dendrimers is therefore a vital step in the designing and engineering of these versatile nanoscopic carriers [88,89] (Fig. 9, Table 2). The different generations of dendrimers having different surface groups (—OH, —NH_2, —$COCH_3$, —COOH, —CN) as well as surface modified dendrimers (folate conjugated, PEGylated, glycosylated etc.) have been separated and identified by analytical methods like high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), NMR, UV–visible spectroscopy, X-ray diffraction etc. Titration and chemical reactions with chemical agents like ninhydrin reagent, reaction with $CuSO_4$ are also used to characterize number of amino groups in dendrimers [88–92]. The presence of tailor-made surface groups facilitates surface modification of dendrimers, which could be further confirmed by reliable analytical methods including infrared spectroscopy (IR), NMR, AFM, X-ray photoelectron spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), matrix-associated laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, size exclusion chromatography, electrospray ionization-mass spectrometry (ESI-MS), vapor phase osmometry (VPO), laser light scattering (LLS) and sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) etc. [92–98]. Various methods of characterization of dendrimers along with description of respective parameters are summarized in Table 2. The principle methods widely used by scientists for characterization of dendrimers are discussed in detail in the following sections.

6.1. IR spectroscopy

Different groups present on dendrimer surface as well as end groups generated by surface engineering can be characterized through infrared (IR) spectroscopy. Jain and coworkers characterized the synthesis of 0.5 G PPI dendrimer [EDA–dendr–$(CN)_4$] by the presence of strong peak of nitrile at 2248.2 cm^{-1}. The conversion of nitrile terminals of 0.5 G PPI dendrimer into amino groups of 1.0 G PPI [EDA–$(NH_2)_4$] dendrimer was confirmed by the presence of major peaks at 3373.9 cm^{-1}, 3294.3 cm^{-1} attributed to the presence of primary amine groups. Synthesis of 5.0 G PPI dendrimer was further confirmed by IR peaks of N–H stretch of primary amine (3396.9 cm^{-1}), N–H bend (1597 cm^{-1}), C–H stretch (2824.2, 2957.5 cm^{-1}), C–H bend (1466.3 cm^{-1}, 1531 cm^{-1}), C–N stretch (1033.2 cm^{-1}, 1081.6 cm^{-1}) [99]. In another study, Jain and coworkers characterized 4.0 G PAMAM through IR spectroscopy by some characteristic peaks such as N–H asym stretch (3473.9 cm^{-1}), N–H sym stretch (3440 cm^{-1}), C–H stretch (2975.9 cm^{-1}), C=O stretch (1731.5 cm^{-1}, 1692.5 cm^{-1}) N–H in plane bending (1599.9 cm^{-1}), C–N stretch (1285.5 cm^{-1}), OCN deformation (630.1 cm^{-1}), C–C bend (1109.6 cm^{-1}, 1052.7 cm^{-1}) [5].

Jain and coworkers synthesized poly-L-lysine dendrimers using sequential protection and deprotection of amino groups of lysine with di-tertiary butyl pyrocarbonate (di-*t*-BOC). Again the protection and deprotection were monitored by IR. Di-*t*-BOC protected 4.0 G lysine dendrimers showed the characteristic peaks of OH stretch (3428.1 cm^{-1}), symmetric CH stretching (2934.8 cm^{-1}), aliphatic CH stretch (2829.9 cm^{-1}, 2722.2 cm^{-1}), —C=O stretch due to ester linkage (1595.8 cm^{-1}), asymmetric and symmetric CH bending (1360 cm^{-1}), CO stretch (1169.1 cm^{-1}), CH bending (1113.1 cm^{-1}), CC stretching (773.4 cm^{-1}), CH rocking (619.5 cm^{-1}) [90]. The detection of functional groups *via* infrared spectroscopy assists in evaluating the formation of different generation of dendrimers as well conjugation of a variety of targeting or imaging ligands at the periphery of dendrimers for targeted drug delivery, or diagnosis of disease, respectively.

6.2. NMR spectroscopy

NMR spectroscopy provides another mean to characterize different groups present on plain as well as engineered dendrimers. Gajbhiye and Jain characterized 5.0 G PPI dendrimer by ^1H NMR spectroscopy using

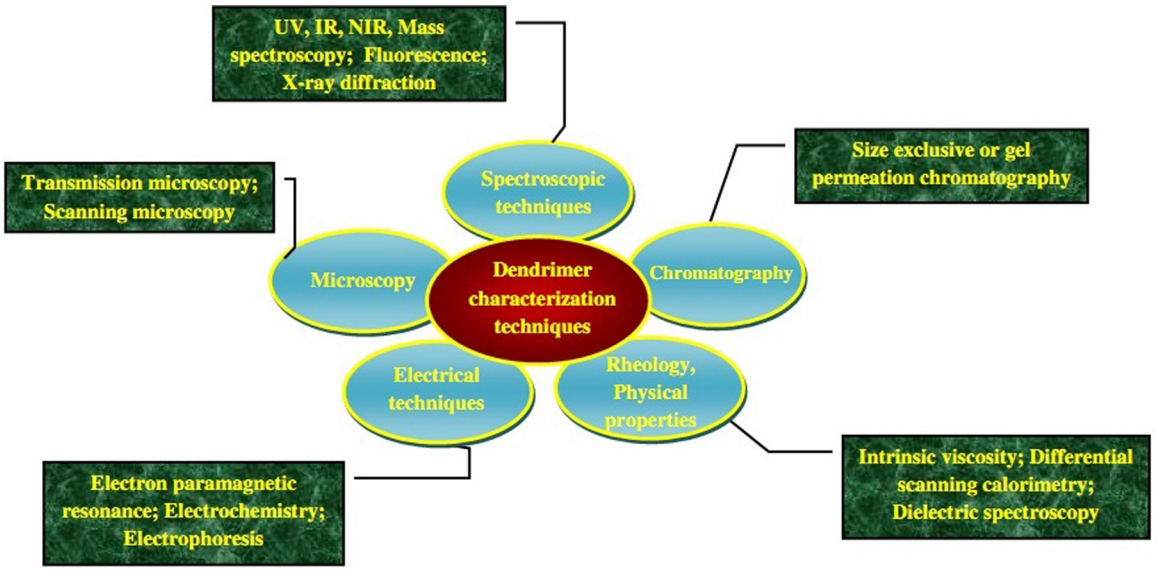

Fig. 9. Methods of characterization of dendrimers.

multiplets between 0.9 ppm and 1.2 ppm and 2.2–2.3 ppm corresponding to methylene (—CH_2—) groups of EDA and multiplets between 2.5 ppm and 2.8 ppm corresponding to —N(CH_3) [100]. Jain and coworkers has also characterized 4.0 G PAMAM dendrimers by ^{1}H NMR spectroscopy. Characteristic multiplets at 2.36–2.39 ppm (triplet) corresponding to R_2CH_2, 2.64–2.69 ppm (triplet) corresponding to —CH_2CO—, 2.770–2.815 ppm (triplet) corresponding to >N—CH_2—CH_2—CO—NH, and 3.026–3.067 ppm (triplet) corresponding to —CH_2NH_2 were observed [5].

6.3. Electron microscopy

Transmission as well as scanning electron microscopy (TEM and SEM) both have been explored to investigate the size, shape, surface morphology of dendrimers. Dendrimer

Table 2
Methods of characterization of dendrimers.

Analytical methods	Characterization parameter
Nuclear magnetic resonance (NMR)	It helps in determining chemical transformation undergone by end groups and hence applicable to structural analysis of dendrimers and step-by-step characterization of synthesis
Infrared spectroscopy and Raman spectroscopy	It ascertains the chemical transformation taking place during the synthesis or surface engineering of dendrimers
UV–visible spectroscopy	It helps in determining the change in chemical structure and synthesis method by detecting chromophores and auxochromes. Also used to test the purity of dendrimers
Fluorescence	It is used to characterize the structure and synthesis of dendrimers having photochemical groups and to quantify defects occurred during the synthesis
Circular dichroism	Characterization of structure of dendrimers having optical activity
Atomic force microscopy	Size, shape and structure
Transmission electron microscopy	
Electron paramagnetic resonance	Surface structure
X-ray diffraction	Chemical composition, size and shape
X-ray photoelectron spectroscopy	Chemical composition and size
Electrochemistry	It gives information about the structure of dendrimers
Electrophoresis	Purity and homogeneity of water-soluble dendrimers
Small-angle X-ray scattering (SAXS)	It gives average radius of gyration (Rg) in solution hence used for determination of average particle size, shape, distribution, and surface-to-volume ratio
Small-angle neutron scattering (SANS)	It gives average radius of gyration (Rg) in solution as well as detailed information about the internal structure of entire dendrimer
Laser light scattering (LLS)	Hydrodynamic radius of dendrimers
Mass spectrometry (FAB-MS, ESI-MS, FT-ICR MS, MALDI-TOF MS)	Determination of molecular mass and some structure information
Size exclusion (or Gel permeation) chromatography (SEC) (GPC)	Molecular weight and size
Intrinsic viscosity	Physical characterization and morphological structure
Differential scanning calorimetry (DSC)	Glass transition temperature (Tg), which is affected by the molecular weight, entanglement and chain-end composition of polymers
Dielectric spectroscopy	Study of molecular dynamics

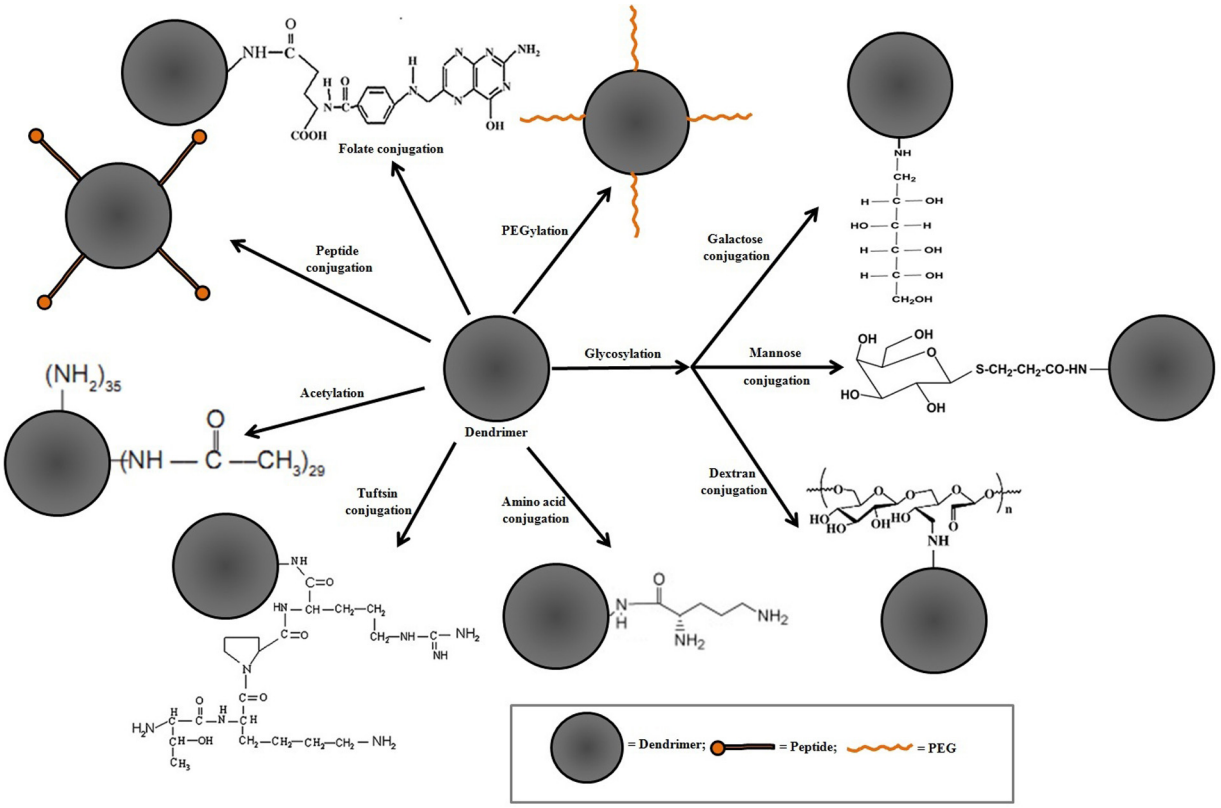

Fig. 10. Different approaches of surface engineering of dendrimers.

surface can be assessed by SEM as Jain and coworkers found a smooth surface of dendrosomes by SEM image [5]. TEM was mainly performed to confirm the size of the synthesized dendrimers [97]. In TEM analysis dendrimers have been found to elicit nanometric size range as evident from various TEM photographs [99,101].

6.4. Size exclusion chromatography

Size exclusion chromatography (SEC) is used to determine absolute molar mass of the substance to be examined and it is also the case with dendrimers. Jain and coworkers determined the absolute molar mass of the PEGylated 5.0 G PPI dendrimers using SEC. The molecular weight was found to be 48,125 indicating that 20 PEG chains were conjugated to one molecule of 5.0 G PPI dendrimers molecule [102]. Hence SEC could be employed to determine not only the molar mass of dendrimers but also the extent of surface conjugation.

6.5. Mass spectrometry

Mass spectrometry has been explored to determine mass of dendrimers, which could help confirming not only the synthesis of dendrimers but also engineering in the dendrimeric architecture. Different methods of mass spectrometry have been explored for characterization of dendrimers. One of these methods is MALDI-TOF mass spectrometry (MALDI-TOF MS). The average mass of the systems is determined from the peaks of parent molecular ion. This method is applicable to higher molecular weight dendrimers, molecular weight is no constraint for this type of mass spectrometry [88,89]. Jain and coworkers determined the molecular weight of EDA-5.0 G PPI–glycine conjugate by MALDI-TOF and the molecular weight of the conjugate was found to be 11663.0 D (4535.0 D more than EDA-5.0 G PPI dendrimer), which helped the scientists in exploring that the increase in molecular weight of conjugate, in comparison to plain dendrimer, corresponded to an average of 60.4666 molecules of glycine conjugated per molecule of EDA-5.0 G PPI dendrimers [103]. In another study, the MALDI-TOF mass spectrum of tuftsin-5.0 G PPI (TuPPI) showed that the tuftsin conjugated PPI dendrimers has a molecular mass of 19,544 D confirming the conjugation of average 24.83 terminal amino groups of PPI dendrimers with tuftsin [104]. In addition to the above discussed methods for the determination of molecular mass; ESI-MS, Fast-atom bombardment-mass spectrometry (FAB-MS), Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), and liquid chromatography–mass spectrometry (LC–MS) have also been employed for determining the molecular weight of different generation of dendrimers, separation and purification of dendrimers [88,89].

Apart from the characterization methods described in this section various other analytical techniques including atomic force microscopy (AFM), confocal microscopy, small-angle X-ray scattering (SAXS), small-angle neutron

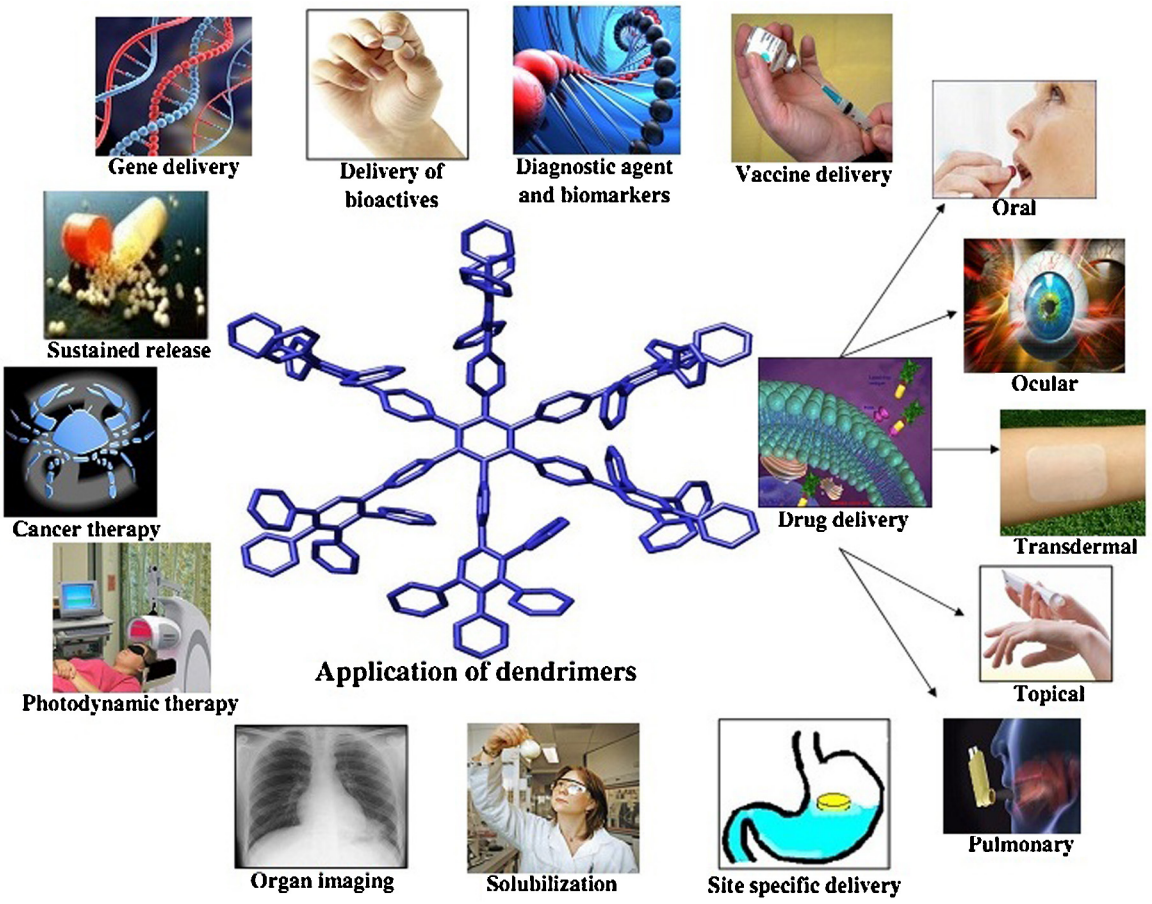

Fig. 11. An overview of dendrimers applications.

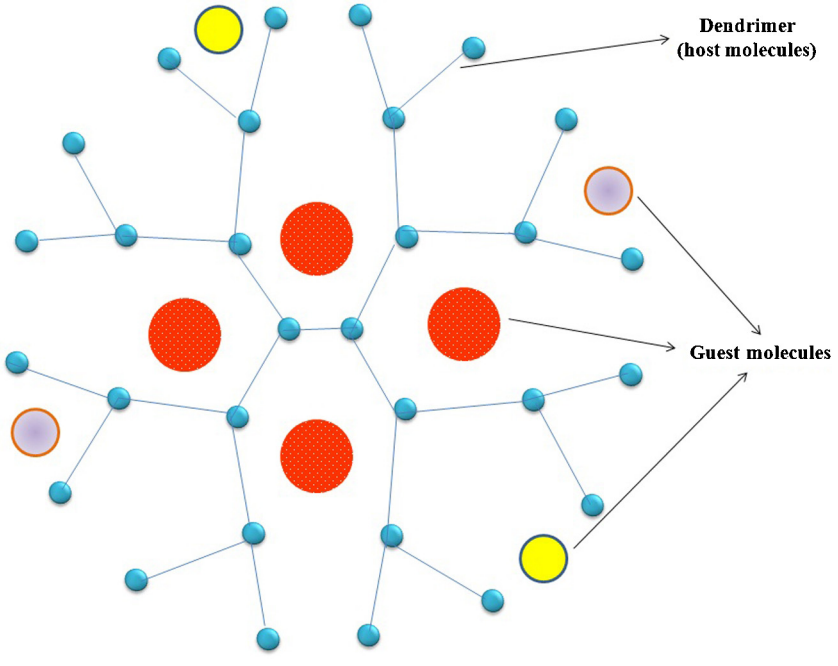

Fig. 12. Host–guest entrapment of a bioactive in the cavities of dendrimer.

scattering (SANS), dynamic light scattering, X-ray photoelectron spectroscopy and X-ray diffraction have been also employed to characterize dendrimers in terms of size, shape, surface structure and chemical composition [89,105]. The surface structure and surface modification of dendrimer could be assessed by electrophoresis as well as polyacrylamide gel electrophoresis (PAGE), agarose gel electrophoresis, capillary zone electrophoresis (CZE). In addition to the surface structure, electrophoresis is also helpful in determining the purity and electrophoretic mobility [88,106]. Other electrical methods such as potentiometric acid-base titration have also been reported for determining the surface modification of dendrimers [89]. The physical parameters of dendrimers like viscosity, glass transition temperature, sedimentation, density etc. have been studied by various methods including viscometer, DSC, absorption spectroscopy, pH and turbidity measurements [88,89,106,107]. Measurement of zeta potential and zeta size has been explored for characterization of dendrimers and dendrimer based formulation like dendrimer–DNA complexes [5,108]. Another method, circular dichroism, has also been adopted to confirm complexation between dendrimers and oligonucleotides as well as alterations in the secondary structure of DNA [5]. DSC has been used to confirm encapsulation of drug molecules into dendrimers via analyzing thermal stability and crystalline transformation over a temperature range [102]. In conclusion, dendrimers have been well characterized for their synthesis, surface modifications, conjugation, physical encapsulation and complexation as well as for various applications in the field of drug delivery *via* a wide range of analytical techniques. However it is essential that the researcher should clearly delineate the purpose, merits and limitations of various analytical tools.

7. Toxicitiy of dendrimers

The fields of medicine and drug delivery have particularly witnessed immense progress after the emergence of nanomaterials such as dendrimers. The well-defined structure, large number of surface groups and nanometric size of dendrimers lead to tremendous advances in intracellular and targeted delivery of drugs. PAMAM dendrimers surface modified with lauryl chains and conjugated with paclitaxel has been found to increase permeability of drug across the cellular barriers and to show the higher cytotoxic potential against human colon adenocarcinoma cell line (Caco-2) [109]. This increase in permeability and cytotoxicity is advantageous in the treatment of diseases like cancer. However dendrimers have been found to exhibhit toxic effects due to their size in the range of nanometers (1–100 nm) and presence of positively charged surface groups in case of cationic dendrimers. The nanometric size and cationic surface groups lead to non-specific interaction of dendrimers with cellular components including cell membrane, nucleus, mitochondria, enzymes, endosomes etc. Although, nanomaterials have brought advances in drug delivery yet the biological and toxicological potential of nanocarriers like nanoparticles, dendrimers, carbon nanotubes is one of the main concern of nanotoxicology [18,28,110].

Toxicity of dendrimers has been primarily explored *ex vivo* with cancer cell lines barring a few reports available on the *in vivo* toxicity potential of dendrimers. The existence of surface $-NH_2$ groups and associated cationic charge on dendrimers confines their applications in drug delivery due to toxicity (Fig. 2). The toxicity of dendrimers is affected by concentration, surface charge, generation, size as revealed through hemolytic toxicity, haematological toxicity, cytotoxicity, immunogenicity and *in vivo* toxicity [18,28]. Cancino et al. evaluated the toxicity of single walled carbon nanotubes (SWCNT), PAMAM dendrimers and PAMAM–SWCNT complexes in mouse myoblast cell line (C2C12). In the results these nanomaterials were found to damage DNA and significantly toxic towards C2C12 cell. Finally the authors concluded that the toxicity of nanomaterials is strongly correlated to their surface charge [110]. Surface charge of dendrimers plays important role in *in vivo* exploration of dendrimers with regards to safety.

Thiagarajan et al. assessed oral drug delivery aptitude of 6.5 G PAMAM dendrimers *via in vivo* oral translocation in CD-1 mice with evaluation of acute oral toxicity and physicochemical disposition and concluded that dendrimers have the potential to permeate gut epithelial barrier [111]. Later, Thiagarajan et al. evaluated the *in vivo* toxicity of PAMAM dendrimers including effect of surface charge and size of dendrimers on the permeability through epithelial barrier and acute toxicity, on oral administration in CD-1 mice. The scientists investigated the positively charged as well as anionic dendrimers for toxicity and determined the maximum tolerated dose (MTD). The MTD for anionic dendrimers was found to be 10 folds higher than for cationic dendrimers. For cationic dendrimers MTD was found to be in the range of 10–200 mg/kg, while anionic dendrimers were found to be tolerable at doses as high 500 mg/kg [112].

Toxicological issues pose most vital limitation in clinical application of dendrimers due to the presence and number of surface amine groups. Recent research has focused on the development of biocompatible dendrimers, which will hasten the era of dendrimer-mediated drug delivery. Apart from designing biocompatible dendrimers, surface engineering presents yet another attractive approach to diminish toxicity of dendrimers; in addition to other beneficial aspects like drug targeting, reduced drug leakage, increased stability, improved pharmacokinetic profile and biodistribution pattern etc. Surface engineering masks the cationic charge of dendrimer surface either by neutralization of charge, for example PEGylation, acetylation, carbohydrate and peptide conjugation; or by introducing negative charge such as half generations of dendrimers (Fig. 10). The modification of PAMAM dendrimers with 4-carbomethoxypyrrolidone groups reduced the toxicity to a significant level. These pyrrolidone modified dendrimers were found to elicit negligible toxicity against Chinese hamster fibroblasts (B14), embryonic mouse hippocampal cells (mHippoE-18) and rat liver derived cells (BRL-3A). The last two decades have witnessed significant development of biodegradable and/or biocompatible, and surface engineered dendrimers resulting into improved therapeutic index. All these efforts resulted into a new class of dendrimer family comprising of biocompatible, biodegradable and surface engineered dendrimers [28,113]. The issues

related to toxicity of dendrimers and the strategies to resolve these toxicities have been discussed in detail by various scientists including our group [28], which could be referred for a deeper insight.

8. Applications of dendrimers

In view of the fact that all the three architectural components; namely the core, internal branching units and the surface groups of dendrimers can be tailored to meet unique properties. These unique properties including unparalleled molecular uniformity, multifunctional end groups and occurrence of numerous internal cavities render dendrimers apposite for potential pharmaceutical applications including various therapeutic and biomedical applications. Applications of dendrimers have been reviewed exhaustively by many scientists [58,91]. Specific applications of dendrimers in drug delivery are summarized in Table 3 and schematically presented in Fig. 11.

8.1. Therapeutic applications of dendrimers

Owing to the exclusive monodispersity, nanometric size range, permeability across the biological membrane and container properties dendrimers serve as appropriate host for the guest molecules either in the interior cavities or on the periphery of the dendrimers (Fig. 12).

8.1.1. Therapeutic activity of dendrimers

Dendrimers are being evolved as topical antimicrobial agents following exploration of effectiveness of polylysine dendrimers against herpes simplex virus (HSV), currently under Phase II clinical trials for its efficacy against vaginal infection. SPL7013 Gel (VivaGel®) developed by Starpharma Pty Ltd (Melbourne, Australia) is a vaginal microbicide for the prevention of HIV and HSV infections [114]. The active ingredient of this Carbopol-based aqueous gel is a dendrimer comprising a divalent benzhydrylamine (BHA) core, four generations of lysine branches with the outermost branches capped with a total of 32 naphthalene disulfonic acid groups that impart hydrophobicity, and a high anionic charge to the dendrimer surface [115]. Success of VivaGel® (Starpharma) gave a philip to the other possible applications of dendrimers.

Wang et al. assessed mechanism of antimicrobial activity of PAMAM dendrimers in guinea pig model of chorioamnionitis against *Escherichia coli* induced ascending uterine infection. The authors attributed the antimicrobial activity to the interaction of polycationic dendrimers with polyanionic lipopolysaccharide present in *E. coli* [116]. Later it was observed that 3.5 G PAMAM dendrimers glycosylated with glucosamine exhibited anti-inflammatory activity by inhibiting complex of lipopolysaccharide, Toll-like receptor 4 (TLR4) and MD-2, which mediates the proinflammatory cytokine responses [117]. This activity of partially glycosylated dendrimers could provide a platform for exploration of dendrimers in the treatment of malignancies, inflammatory diseases as well as infectious diseases.

8.1.2. Solubilization

The poor solubility *vis a vis* hydrophobicity of most of the drug molecules limits their applications. It is the major constraint in the development of a safe, effective and stable formulation. Various conventional as well as novel methods have been employed to overcome solubility problems. Dendrimers have been explored by various scientists as a promising candidate for solubilization of bioactives having different therapeutic activity like anticancer, antimalarial, antiviral, antitubercular, antimicrobials, NSAIDs and antihypertensive etc. [50]. Dendrimer-mediated solubilization is affected by various factors including generation size, concentration of dendrimers, pH, core, internal branching units, surface groups as well as temperature. Micellar solubilization, ionic interactions, hydrophobic interactions as well as hydrogen bonding are the main mechanisms, which are sought to be accountable in dendrimer-mediated solubility enhancement. Solubilization efficiency of dendrimers can be easily modified by tailoring core, branching unit and surface functionalities or engineering surface of dendrimers with hydrophilic moieties [118–120]. In a study with polypropylene oxide cored PAMAM dendrimers it was observed that the solubilization efficacy of dendrimers is proportional to their generation and concentration. The study was performed with NSAIDs including Ketoprofen, Ibuprofen and Diflunisal and authors concluded that dendrimers are effective solubility enhancer for NSAIDs [121]. Dendrimers emerge out as outstanding carrier in respect of bioavailability enhancement of chemical entities.

8.1.3. Dendrimers in transdermal drug delivery

Recently dendrimers have been investigated for transdermal delivery of drugs due to the two facts: (i) the presence of hydrophobic moieties in most of the drugs resulting in poor water solubility, which restricts the entry of drugs in the biological compartments, and (ii) high water solubility and biocompatibility of most of the premeditated dendrimers.

Dendrimers have been found effective in efficient transdermal delivery of drugs with improved pharmacokinetic profile [59]. Dendrimers have been investigated with different non steroidal anti-inflammatory drugs (NSAIDs) for transdermal delivery. Jain and coworkers investigated the 4.0 G PAMAM dendrimers with amino and hydroxyl terminal and 4.5 G PAMAM dendrimers for transdermal delivery of a model drug, indomethacin. In the *in vivo* pharmacokinetic and pharmacodynamic studies with Wistar rats, a significant increase in concentration of indomethacin in blood was observed in case of PAMAM dendrimers-mediated delivery of indomethacin, in comparison to that observed with pure drug suspension [122]. Later, Cheng and coworkers developed the conjugate of ketoprofen and diflunisal with 5.0 G PAMAM dendrimers. In the *in vitro* permeation studies with excised rat skin, ketoprofen–dendrimer and diflunisal–dendrimer complex displayed the 3.4 and 3.2 times higher permeation rate compared to ketoprofen and diflunisal dispersion in saline, respectively. In the anti-nociception effect studies on mice, reduction in the writhing activity was observed during 1–8 h, on transdermal administration of ketoprofen–dendrimer complex, while with ketoprofen

Table 3
Therapeutic applications of dendrimers.

Application	Dendrimers	Bioactive(s)	Outcome	References
Solubilization	PPI dendrimers	Amphotericin B Famotidine Indomethacin	Dendrimers can enhance solubility of acidic, basic as well as amphoteric drugs (increase in solubility clearly depends on chemical nature of drug and pH)	[50]
Enhanced cellular uptake	Mannosylated PPI dendrimers	Efavirenz	12 and 5.5 times increase in cellullar uptake of efavirenz was obtained with mannosylated PPI dendrimers after 48 h in comparison to free drug and t-Boc–glycine conjugated PPI dendrimers, respectively	[153]
Biocompatible drug carrier	Mannosylated PPI dendrimers	Rifampicin	A biocompatible formulation was obtained	[196]
Drug targeting	Mannosylated PPI dendrimers	Rifampicin	Site-specific delivery to the alveolar macrophages was achieved	[196]
	PPI (5.0 G), mannosylated and lactosylated PPI dendrimers	Radioactive technetium (sodium pertechnetate; $^{99}mTcO_4^-$)	In biodistribution studies in female Balb/c mice, all formulations were found to accumulate in liver but dendrimers with mannose and lactose terminal retained for longer time in liver. Concluded that carbohydrate-coated dendrimers can selectively deliver the bioactives to liver	[87]
	Dextran conjugated PPI dendrimers	Doxorubicin HCl	Enhanced uptake by A549 cancer cell lines was observed	[93]
	Folate conjugated dendrimers	Methotrexate	Folate conjugated dendrimers enter the cancer cell by receptor-mediated endocytosis	[136]
Sustained release	Mannosylated PPI dendrimers	Lamivudine	Mannosylated dendrimers prolonged the release rate up to 144 h	[148]
	Dextran conjugated PPI dendrimers	Doxorubicin HCl	Developed formulation showed sustained release profile compared to free drug	[93]
Reduction in toxicity	Galactose conjugated poly-L-lysine dendrimers	Chloroquine phosphate	Galactosylated dendrimers showed the negligible hemolytic toxicity with reduction in toxicity of chloroquine phosphate	[90]
	Mannosylated PPI dendrimers	Efavirenz	Mannose conjugated dendrimers showed negligible cytotoxicity in human hepatoma (HepG2) cell lines and very little hemolytic toxicity, respectively i.e. $2.8 \pm 0.04\%$ and $5.3 \pm 0.03\%$ at a concentration of 1 mg/ml	[197]
	Dextran conjugated PPI dendrimers	Doxorubicin HCl	16.7% hemolysis was observed with drug (0.1 μM) whereas 12.3% hemolysis was observed with drug loaded dextran conjugated PPI dendrimers	[93]
MRI contrast agents (contrast or imaging agent)	Poly(2,2-bis[hydroxymethyl]propanoic acid) (5.0–7.0 G)	^{99m}Tc	MRI agent for kidney/bladder in Nude Copenhagen rats	[198]
	PAMAM (4.0 G)	(1B4M-Gd)64	MRI agent for kidney in Nude mice	[199]
	PAMAM-cystamine (6.0 G)	GD-DO3A	MRI agent for breast cancer in Nude mice	[200]
	PAMAM (6.0 G)	(1B4M-Gd) 256	MRI agent for breast cancer in mice	[201]
	PAMAM (6.0 G)	G6-Cy(5.5)1.25(1B4-Gd)145	MRI and fluorescein imaging agent for sentinel (mammary) lymph nodes in mice	[202]
	PAMAM (8.0 G)	(1B4M-Gd)1024	MRI agent for sentinel (mammary) lymph nodes in mice	[155]
	PAMAM (6.0 G)	(1B4M-Gd)256	MRI agent for sentinel (mammary) lymph nodes in mice	[155]
Immunotherapy (antibody conjugate)	PAMAM dendrimers (5.0 G)	60bca and J591	Carrier	[145,203]
	PAMAM	Antibody	Binding (Anthrax detection) [Alert Ticket™ (by US army)]	[204]
	PAMAM	Monoclonal sheep antibody	Carrier [Stratus® CS Acute Care™ Anti-proBNP (pBNP) (Siemens Healthcare)]	[205]

Gene transfection	PPI-DAB dendrimers	DNA and Cucurbituril	Carrier	[206]
	PAMAM dendrimers	DNA	Complexation	[207–209]
	PAMAM-Arginine dendrimers			[210]
	PAMAM dendrimers	Oligonucleotides (linear and anionic)	Physical mixing (commercially available as SuperFect®)	[58]
	Priostar-PAMAM	DNA/SiRNA	Complex	[132]
	PAMAM (4.0 G)	Oligo-DNA	Complex	[210]
	4.0 G PAMAM	siRNA and DOX	Effective intracellular accumulation of siRNA with co-delivery of drug was obtained with developed system	[211]
As a sensor in liquid media	Thiol 3.0 G PPI dendrimer coated with CdSe quantum dots		Developed system displayed chemical sensor activity for heavy metal in liquid media	[212]
Glucose biosensor	4.0 G PAMAM		Developed biosensor was successfully applied for the glucose analysis in beverages	[213]
As a fluorescent sensor	1.0 G PPI		Synthesize new dendrimer [blue fluorescent poly(propyleneamine) dendrimer whose periphery is modified with a 4-(N,N-dimethylaminoethyloxy)-1,8-naphthalimide] allows development of a new selective fluorescent sensor for Fe^{3+}	[214]
Intracellular drug targeting delivery	Mannosylated PPI dendrimers	Rifampicin	Delivery of rifampicin to alveolar macrophage	[215]
Complexes	PAMAM (0.0–3.0 G)	Furosemide	Complexation	[216]
	Mannosylated PPI dendrimers (5.0 G)	Lamivudine		[148]
	Folate conjugated PAMAM dendrimers	Indomethacin		[217]
Complexation	PAMAM (4.0 G)	Azithromycin	PAMAM–Azithromycin complex delivered azithromycin effectively for treatment of chlamydia infection	[218]
	PAMAM (4.0 G)	Erythromycin	PAMAM–Erythromycin conjugates showed high drug loading, improved solubility and reduced local periprosthetic inflammation in a sustained manner	[151]
	PAMAM		Manganese complexes of polystyrene-supported PAMAM dendrimer were found to be efficient and reusable catalysts for the oxidation of secondary alcohols to ketones under mild reaction conditions	[219]
	2.0 G and 3.0 G PPI	NiFe nanoalloy	Synthesize dendrimer (2.0 G and 3.0 G PPI) capped NiFe nanoalloy exhibited photoluminescence properties	[220]
Encapsulating agents	Core PAMAM dendrimers – caprolactone – polyethylene glycol	Etoposide	Micelles	[218]
	PEG-mesylate	Indomethacin		[221]
	PAMAM	Methotrexate	Liposomes	[6]
Delivery of anticancer bioactives	PPI	Paclitaxel	Conjugation of surface amino groups of PPI dendrimers with folate, galactose and dextran	[92]
	4.0 G PAMAM	DOX	Synthesis of collagen peptide-modified dendrimer attached doxorubicin DOX via a pH-degradable linkage as a polymer prodrug	[222]
Delivery of anti-HIV drug	4.0 G PPI	Zidovudine	Sialic acid conjugated-Mannosylated PPI (SMPPI) dual ligand dendritic system showed enhanced biocompatibility and site-specific delivery of Zidovudine	[141]

Table 3 (*Continued*)

Application	Dendrimers	Bioactive(s)	Outcome	References
Cardiac function	4.0 G PAMAM	siRNA	Developed oligo-arginine-conjugated tadpole dendrimer found to be non-cytotoxic and efficient non-viral delivery system for siRNA in cardiac tissue, and the delivery of siRNA against AT1R by the dendrimer was able to preserve cardiac function after ischemia-reperfusion injury in rats	[223]
Reduction of 4-nitrophenol	4.0–6.0 G PAMAM-OH and PAMAM-NH_2		Cu, Ag and Au dendrimer-encapsulated NPs exhibited good activity for reduction of 4-nitrophenol to 4-aminophenol	[224]
Photodynamic therapy	3.0 G PAMAM		3.0 G PAMAM dendrimer-grafted porous hollow silica NPs have been successfully fabricated as photosensitive drug carriers for PDT	[225]

dispersion writhing was reduced during 4–6 h post transdermal administration. The authors concluded that dendrimer based transdermal delivery system of NSAIDs could be explored as an attractive approach for treating various ailments [123]. Overall, dendrimers hold promise in transdermal drug delivery although available reports are inadequate.

8.1.4. Dendrimers in oral drug delivery

Oral route for administration of anticancer agents is preferred because this route is convenient to patients, requires reduced cost of administration and also facilitates the use of more chronic treatment regimens. But the problems like low aqueous solubility and poor permeability across the biological membrane associated with drugs limit the application of oral route [124,125]. Dendrimers have been investigated for the oral delivery of various drugs with promising results. Plain as well as surface modified dendrimers have been found to increase the transepithelial permeability. Jevprasesphant et al. studied the permeation of PAMAM dendrimers and surface modified PAMAM dendrimers across the Caco-2 cell monolayers by measuring the TEER and [^{14}C] mannitol apparent permeability coefficient at 4 °C and 37 °C in both directions i.e. apical to basolateral and basolateral to apical directions in the presence and absence of ethylenediamine tetraacetic acid (EDTA) and colchicines. The researchers concluded that PAMAM dendrimers and surface modified dendrimers with lauroyl groups could efficiently traverse epithelial monolayers via paracellular and transcellular pathways [126]. Later, propranolol–PAMAM dendrimer conjugate was investigated for transport across Caco-2 cell monolayers and it was observed that the conjugate could reduce the effect of P-glycoprotein on intestinal absorption of propranolol. Hence it could be concluded that dendrimers can bypass P-glycoprotein efflux transporter and can facilitate the oral administration of drugs [127]. Najlah et al. investigated the oral delivery potential of prodrug of naproxen based on PAMAM dendrimers. The low aqueous solubility of naproxen hinders its oral bioavailability. In this study authors investigated the transepithelial permeability of naproxen–dendrimer conjugates and stability of these conjugate in 50% liver homogenate and 80% human plasma. Two different linkers i.e. lactate ester and diethylene glycol were used to link drug to dendrimers and these linkages showed considerable influence on the stability of conjugate. Conjugates with lactate ester linker were more stable in plasma and illustrate the slow hydrolysis in liver homogenate whereas diethylene glycol linker based conjugate demonstrated the high chemical stability with quick release of drug in plasma and liver homogenate. Finally authors concluded that (i) dendrimer based conjugate of naproxen could enhance the oral bioavailability, and (ii) conjugate based on lactate ester linker may serve as promising candidate for controlled release [128]. Conjugate of anticancer drug 7-ethyl-10-hydroxy-campothecin (SN38) with 3.5 G PAMAM dendrimers showed improved oral bioavailability with reduced toxicity [129]. Sweet et al. investigated the effect of PEGylation on the anionic PAMAM dendrimers with respect to toxicity as well as transepithelial

transport and cellular uptake. Two generations of PAMAM dendrimers 3.5 G and 4.5 G were PEGylated and it was observed PEGylation of 3.5 G PAMAM dendrimers resulted in decreased cellular uptake and transepithelial transport whereas PEGylated 4.5 G dendrimers showed increased uptake with decreased transport. This was thought to be due to the reduction in the opening of tight junction by PEGylated dendrimers. Finally authors concluded that PEGylated dendrimers could be explored in oral delivery when drug conjugation and drug release dictate increased surface functional groups [130].

8.1.5. Dendrimers in ocular delivery of bioactives

The treatment of ocular disorders necessitates the topical application of bioactives nevertheless intraocular delivery suffers from poor bioavailability problems due to elimination of formulation by (i) tear turnover, and (ii) nasolacrimal duct-mediated drainage of fluid in excess. Thus the development of formulation, which can overcome these limitations of ocular route, emerges as an arena for extensive research. In addition to this, formulation intended to be applied by ocular route should be non-sensitizing, non-irritating, isotonic, biodegradable and biocompatible with good retention within the eye [59,131,132]. Dendrimers have been explored for the ocular delivery of bioactives. Vandamme and Brobeck, found the PAMAM dendrimers with carboxyl or hydroxyl end functionalities, to increase the retention of pilocarpine within the eyes. Thus this study supports the applicability of dendrimers via ocular route [133].

8.1.6. Dendrimers in pulmonary delivery

Dendrimers have been evaluated as a carrier for pulmonary delivery of a low molecular weight heparin, enoxaparin. In this study 2.0 G, 2.5 G and 3.0 G of PAMAM dendrimers were assessed for pulmonary absorption of enoxaparin, which was estimated indirectly by determining the anti-factor Xa activity and analyzing the deep vein thrombosis preclusion efficacy in a rodent model. In these studies, cationic charged 2.0 G and 3.0 G dendrimers were found to increase the relative bioavailability of enoxaparin by about 40% without any adverse effect on mucocilliary transport rate, and without producing the severe damage to lung tissues, whereas negatively charged dendrimers with carboxyl end groups (2.5 G) did not influence the bioavailability. So dendrimer with surface cationic charge can serve as promising vehicle for pulmonary delivery of bioactives [59,134]. Applications of various dendrimers via different routes are briefly summarized in Table 4. Mignani et al. have recently reviewed various routes including oral, transdermal, ocular and transmucosal for exploration of dendrimers as drug delivery system [135].

8.1.7. Dendrimers in targeted drug delivery

Currently treatment of ailments is aimed at increasing the therapeutic efficacy of bioactives by selective delivery to target site. This tactic increases the therapeutic index of drug by increasing its efficacy and reducing its adverse effects. Dendrimers can easily provide the targeted delivery of medicaments by passive as well as active targeting, which is achieved by engineering the branching units and surface groups of dendrimers. This approach is particularly effective in treating the fatal disorders like cancer and diseases caused by parasitic infection. The target site is well-defined in these ailments and hence efficiently engineered, site-specific carriers can be designed.

Dendrimers have emerged as versatile carrier in this regard because of their well-defined architecture, monodispersity, tailor-made surface groups. These properties make dendrimers useful in targeted delivery of bioactives. One of the most explored examples in this context is folate conjugated dendrimers for targeting anticancer bioactives to tumor. Since the folate receptors are over-expressed on the surface of different types of cancer cells such as ovarian cancer, breast cancer etc., hence folate conjugated dendrimers can efficiently target anticancer bioactives to cancer cells [136,137]. In a study, folic acid was conjugated to PAMAM dendrimers followed by coupling with anticancer drug methotrexate (MTX) and evaluated by biodistribution studies and confocal microscopy in immunodeficient mice enduring human KB carcinoma. In the biodistribution studies folic acid conjugated dendrimers showed three times higher accumulation in the tumor cells after 24 h compared to those conjugated without folic acid. Confocal microscopy and flow cytometric analysis further confirmed the results of biodistribution studies [138]. Thomas et al. conjugated the CD14 and prostate-specific membrane antigen (PSMA) antibodies to 5.0 G PAMAM dendrimers with fluorescein imaging tag. In the flow cytometry and confocal microscopy studies the conjugate was found to bind specifically to antigen-expressing cells in an affinity comparable to free antibody. The binding affinity was time- and dose dependent [139]. Choi et al. designed folic acid and FITC conjugated PAMAM dendrimers for tumor targeting and imaging, respectively, followed by linking of complementary oligonucleotides for cell specific binding and internalization. Hence dendrimers provide a template with which it is possible to conjugate more than one ligand for different purposes [140].

The well-defined structure of dendrimers provides the opportunity for multifunctional engineering. Jain and coworkers successfully designed dual ligand-conjugated PPI dendrimers i.e. sialic acid conjugated mannosylated dendrimers for dual targeting of anti-HIV drug Zidovudine. This dual conjugated system was found efficient in increasing the biocompatibility as well as targeted delivery of antiviral drug [141].

8.1.8. Reduction of toxicity

Although dendrimers with cationic surface groups cause cytotoxicity and hemolytic toxicity yet their toxicity can be alleviated by modification of surface groups with biocompatible ligands such as PEG, acetyl group, carbohydrates, amino acids and peptides etc. The surface engineering of dendrimers results in biocompatible dendrimers as well as reduces the toxicity of some cytotoxic and hemolytic bioactives [28,131].

Dendrimers show the surface charge-, concentration- and generation-dependent cytotoxicity. The permeability profile of dendrimers is also related to its surface charge. Cationic dendrimers have been found to be more toxic (hemolytic as well as cytotoxic) and more permeable than

Table 4
Summary of applications of dendrimers via different routes.

Route of drug delivery	Dendrimer	Limitations of conventional formulations	Bioactive	Advantages of dendritic formulation	References
Parenteral	PAMAM (4.0 G)	–	N-acetyl cysteine	Carrier	[226]
	Poly-L-lysine (PEG 1000) (3.0 G and 4.0 G)	–	Chloroquine phosphate	Encapsulating agent	[90]
Transdermal	PAMAM dendrimers	1. Gastrointestinal side effects including dyspepsia, gastrointestinal bleeding (on oral administration) 2. Renal side effects (on oral administration) 3. Poor rates of transcutaneous delivery due to barrier posed by skin	NSAIDs (Ketoprofen, Diflunisal)	1. Complex of NSAIDs (Ketoprofen, Diflunisal) with dendrimers resulted in improvement of drug permeation through skin 2. 3.4 and 3.2 times higher permeation observed for Ketoprofen and Diflunisal complexed with dendrimers, respectively	[123]
	PAMAM dendrimers with amino and hydroxyl terminals		NSAIDs (Indomethacin)	Higher concentration of Indomethacin was observed in the blood of Wistar rats after application of dendrimer based transdermal formulation to abdominal skin	[122]
Oral	PAMAM dendrimers	Low solubility and/or poor permeation across the biological membrane hinders the oral absorption. Removal of drug by P-gp efflux transporter	–	1. Dendrimers can cross epithelial monolayers by pathways, paracellular and transcellular 2. Drug dendrimer conjugate can reduce the effect of P-gp on intestinal absorption of drug administered orally 3. Dendrimer based drug conjugate can significantly enhance the oral bioavailability	[126]
			Propranolol		[127]
			Naproxen		[128]
Pulmonary	PAMAM dendrimers	Pulmonary administration of some drugs results in adverse effect on mucocilliary transport rate and extensive damage of lungs	Enoxaparin (low molecular weight heparin)	Considerable increase in rate and extent of absorption observed with dendrimers having cationic surface groups	[134]
Ocular	PAMAM dendrimers with hydroxyl/carboxyl peripheral groups	Ocular administration of drugs is limited by two factors (i) poor retention of dosage form for the ocular region, and (ii) patient inconvenience	Pilocarpine	Increased retention of pilocarpine within the eyes	[131,133]
Colon delivery	3.0 G PAMAM	–	5-Amino salicylic acid	Carrier	[227]
Topical delivery	5.0 G PAMAM	–	Nifedipine	Solubility and permeation enhancer	[118]
	2.0–6.0 G PAMAM	–	5-Fluorouracil	Permeation enhancer	[228]
	4.0 G Lysine based dendrimers	–	SPL7013 [Poly(L-lysine)-based dendrimer with naphthalene disulfonic acid surface groups]	Therapeutic agent (anti-HIV agent)	[229,230]

the anionic and neutral dendrimers (Fig. 2). Designing of biocompatible and biodegradable dendrimers either by synthesizing dendrimers from biocompatible units (peptides, amino acids, carbohydrates etc.), or modifying the surface of cationic dendrimers with biocompatible ligands (PEGylation, acetylation, glycosylation etc.), will facilitate reduction in toxicity [28,91].

8.2. Biomedical applications

Owing to well-defined size and structure, dendrimers attracted attention of researchers for biomedical applications like controlled delivery of bioactives, gene transfection, and as imaging and diagnostic agents. The nanometric size, extensive branching, tailor-made

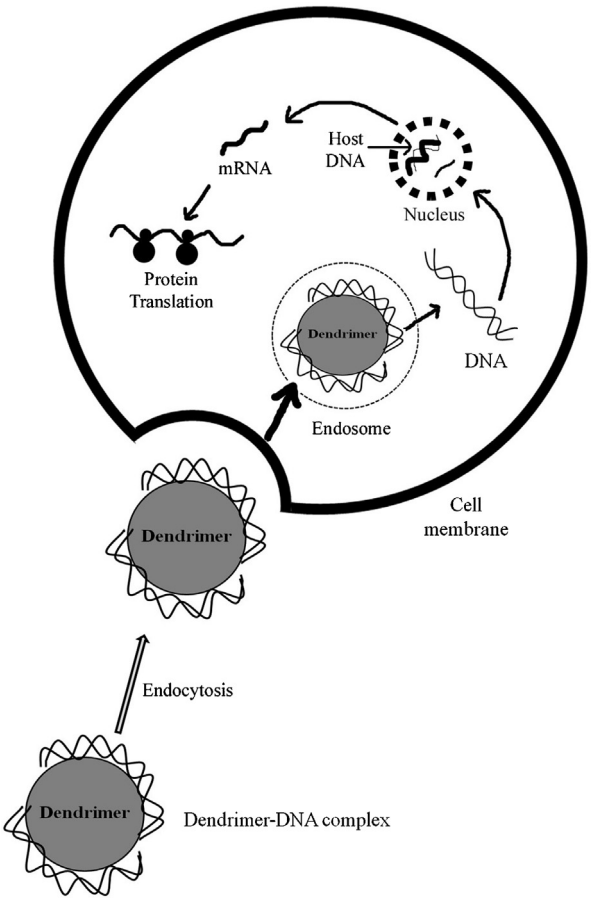

Fig. 13. Dendrimer-mediated gene transfection.

surface groups, monodispersity and excellent stability are some unique features, which make dendrimers particularly finest candidate for application in biomedical field. Various scientists have reviewed different biomedical applications of dendrimers including gene delivery, diagnostic agent, vaccines etc. [58,142].

8.2.1. Dendrimers in gene delivery

Plethora of research is now available on the applicability of dendrimers in gene transfection, particularly amino terminated dendrimers like PPI, PAMAM, arginine and ornithine conjugated dendrimers. These amino terminated dendrimers enhance the transfection efficiency into nucleus by endocytosis [58,143–145]. Dendriplexes i.e. complexes of dendrimers and nucleic acids showed good transfection efficiency and hence explored for the delivery of genetic materials including oligonucleotides, genes, aptamers, siRNA etc [146]. Dendrimers with structural flexibility and hyperbranched architectures are suitable candidates for gene delivery operations owing to formation of more compact complexes with DNA, attributed to enhanced flexibility of dendrimers. The mechanism of genetic materials delivery to the nucleus and translation assistance is depicted in the Fig. 13. Basically in the first step dendrimer–DNA complex is taken up by cells by endocytosis, and consequently endosomal destabilization of electrostatically assembled dendrimer–DNA complex takes place, which is followed by release of DNA. Then this DNA is taken up by nucleus where it replicates with host DNA. Subsequently transcription takes place, and as a result, mRNA is released as biosignal, which is followed by translation of target protein [147]. All these steps are depicted in the Fig. 13.

8.2.2. Dendrimers as nanoscale containers (nano-scaffolds)

The tailor-made surface groups, interior branching and core epitomize three components of dendrimers, which can function as carrier for bioactives. Particularly the interior cavities of dendrimers are proficient at encapsulation of drugs as guest molecules, while dendrimers serve as host (Fig. 12). The surface groups of dendrimers, which are exposed to exterior, are predominantly imperative for targeted delivery [45]. PPI dendrimers have been modified at surface via amino acids to design dendritic boxes. Dendritic boxes are characterized by the formation of dense, hydrogen bonded surface shells with solid state character with excellent entrapment properties. It was observed that encapsulation efficiency of these molecules depends on the two factors; first one being the amount, shape as well as size of internal cavities accessible in the dendrimers, and second is the shape and size of the molecule to be encapsulated in the dendrimers. For example, it was observed that a PPI dendrimer with 12 small cavities and four large cavities can encapsulate 8–10 molecules of bioactive with small size like p-nitrobenzoic acid, which were incapable of leaking out. In contrast, only four molecules of large bioactives (e.g. Rose Bengal dye) can be accommodated into these dendrimers [7,58].

8.2.3. Intracellular delivery of bioactives

One of the most promising applications of dendrimers is their ability to deliver the molecular cargo at intracellular level. They have been successfully utilized by various scientists for intracellular delivery of medicaments to achieve intracellular level targeting. Targeting of antiviral drugs to the macrophages has been achieved with dendrimers surface engineered with mannose to accomplish mannose receptor-mediated endocytosis (Fig. 14) [148]. Enhanced uptake of dendrimers with consequent release of anticancer agent into cancer cells has been reported by many scientists [92,93].

To achieve intracellular delivery of bioactives it is necessary to minimize extracellular leakages by preventing the non-specific interaction of dendrimers with systemic circulation as well as ensuring that the drug loaded nanometric dendritic system will not be cleared off very rapidly from the systemic compartment. Conjugation of PAMAM dendrimers with methylprednisolone resulted into improved uptake by human lung carcinoma epithelial cell line. In this study, the activity of dendritic complex was found to be comparable to free drug [149]. In case of some dendrimeric complexes of drug, it has been also observed that the drug–dendrimer complex is rapidly internalized by the target cells, although drug was released slowly over a prolonged period of

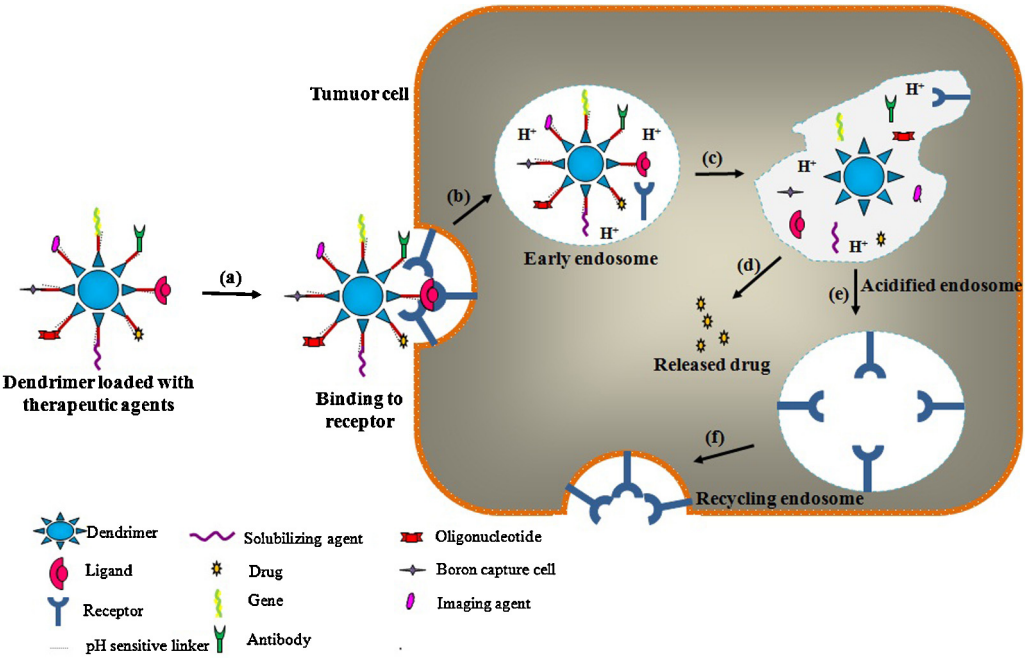

Fig. 14. Schematic representations of pH-responsive bioactive release from targeted dendrimeric conjugate possessing pH sensitive linker: (a) receptor association of ligand-conjugated bioactive-loaded dendrimers, (b) endosomal uptake of conjugates, (c) rupture of acid-sensitive linkage between dendrimer and bioactive ensuring selective endosomal release (at pH < 7), (d) release of drug at targeted site, (e) formation of recycling vesicles, and (f) receptor regeneration.

time. Kolhe et al. observed that while ibuprofen complexed PAMAM dendrimers was rapidly taken up by A549 cells. The covalently linked ibuprofen showed the sustained release. It was concluded that dendrimers as well as surface engineered dendritic nano-architectures could be successfully exploited for intracellular level of targeting of medicinal substances [150]. Bosnjakovic et al. developed erythromycin conjugated PAMAM dendrimers for treatment of periprosthetic inflammation *via* targeted and sustained intracellular delivery to macrophages exploiting inflammation inhibitory potential of erythromycin and targeting ability of dendrimers. Antibiotic erythromycin was conjugated to dendrimers by way of ester bond and activity was evaluated in RAW 264.7 macrophage cell lines. The conjugate was found to improve activity of erythromycin against periprosthetic inflammation with high drug payload, improved solubility and sustained release [151]. Similarly, PAMAM dendrimer–azithromycin conjugate was found to be efficient in intracellular delivery of azithromycin to treat *Chlamydia trachomatis* infection [152].

8.2.4. Microvascular extravasation

Owing to the nanometric size and lower molecular weight, dendrimers show the extravasation propensity, which is defined as the movement of molecules from the blood circulatory system across the endothelial lining of capillary walls into the neighboring interstitial tissues [91]. Extravasation is essential for effective targeted delivery of medicinal substances. Kitchens et al. inspected the extravasation of different generations of PAMAM dendrimers through the microvascular endothelium and concluded that the extravasation time is exponentially proportional to the size and molecular weight of dendrimers in following order 0.0 G < 1.0 G < 2.0 G < 3.0 G < 4.0 G. Time for extravasation was in the range of 143.9–422.7 s [153]. Microvascular extravasation is particularly important for passive tumor localization of anticancer bioactives and diagnosis of cancer via MRI contrast agent etc. [154,155].

8.3. Diagnostic applications

The unique morphology and distinct characters of dendrimers makes them promising candidate for diagnostic applications. Dendrimers have been used efficiently as imaging agent, in radiotherapy, as X-ray and MRI contrast agent as well as molecular probes [84,156].

Dendrimers linked to various ligands have been used for molecular detection, separation, radiotherapy and as imaging agent. Wu et al. exploited the antibody linked metal–chelate–dendrimers for radioimmunotherapy and imaging purposes [157]. Similarly FITC tagged PAMAM dendrimers has been investigated for determining the cellular uptake [158]. The spectrum of the diagnostic applications of dendrimers is likely to broaden in near future.

8.3.1. X-ray contrast agent

Substances of high molecular weight are preferred over low molecular weight as X-ray contrast agent as the low molecular weight substances equilibrate readily within the extracellular fluid and intravascular compartments of the body. Hence high molecular weight contrast agents are used to achieve X-ray image with high resolution to detect disease lesions or view organs; for example

kidneys, bones, fracture, arteriosclerotic vasculature, tumors and infarcts etc. Various groups of scientists are exploring dendritic polymers as X-ray contrast agent, by designing organometallic (Bi and Sn) complexes of dendrimers, dendritic nanoparticles with heavy isotopes of iodine, dendrimers with PEG cores etc., for microvascular quantification of various diseases as well as in angiography and computed tomography imaging [157,159,160]. In most of the studies, the dendrimers-based contrast agent was found to be advantageous on account of one or more of the following characteristics; high iodine content, prolonged intravascular enhancement, high X-ray attenuation, osmolality, good chemical stability, high water solubility and hydrophilicity [161].

8.3.2. Molecular probes

The unique properties and architectural specificity of dendrimers makes them interesting candidate as molecular probes. To exploit dendrimers as molecular probe, one of the strategies is generation of an integrated dendrimer based molecular probe by immobilizing the sensor units on the surface of dendrimers. One example of this strategy is the designing of a dendrimer with Pt coordination complexes as end groups having high sensitivity to sulfur dioxide (SO_2). On exposure to SO_2, due to electronic absorption, the square-planar Pt II complex changes to five-coordinate SO_2 adduct [162]. Dendrimers have also been modified or surface functionalized to design molecular probe for avidin, DNA, amino alcohols and pH etc. [163–167].

8.3.3. MRI contrast agent

X-ray imaging contrast agents are also essential for Magnetic Resonance Imaging (MRI). MRI is a distinguished non-invasive technique for the diagnosis of various diseases, which is based on different relaxation time of protons in different tissues. Low toxicity, biocompatibility and high relaxivity are the desirable qualities for MRI contrast agents. The most widely used contrast agent in MRI for clinical application is Gd (III) ions, which unfortunately are highly toxic due to strong affinity for serum protein. Hence a chelate, which is the complex of diethylene triamine pentaacetic acid (DTPA), is also being used clinically as contrast agent [156,168]. However as discussed under X-ray contrast agent, high molecular weight MRI contrast agents are preferred over low molecular weight contrast agents because the later diffuse readily from blood vessels into interstitial space followed by rapid clearance from the body. To combat this problem several complexes of Gd with high molecular weight compounds like albumin, dextran, polylysine and dendrimers are being explored [169–171]. Dendrimer based DTPA complexes have shown the potential to improve upon all the limitations of previously used MRI contrast agents [155,156].

9. Surface engineering of dendrimers

The major constraints to the biological and biomedical applications of dendrimers are related to hemolytic toxicity, immunogenicity, RES uptake, stability, hydrophobicity, drug leakage etc. Dendrimers may also interact effectively with the components of cell such as plasma membranes, cell organelles, and proteins such as enzyme etc., due to their nanometric size. Most commonly used dendrimers like PPI, PAMAM and PLL exert considerable *in vitro* cytotoxicity due to their surface cationic groups [29,172]. Only if these problems are resolved then dendrimers may be one of the better, if not best, options among the others nanocarriers. There are various strategies available for surface engineering of dendrimers, such as PEGylation, coating with other carriers, attachment of ligands etc. [131] which can generally overcome these limitations (Fig. 10).

PEGylation, conjugation or linking with the dendritic system with PEG additionally increase the solubility of hydrophobic drugs. PEG enjoys various advantages like non-toxic, non-antigenic, non-immunogenic, high solubility in water and FDA approval (Fig. 15). The PEG–drug conjugates have several advantages such as decreased degradation by metabolic enzymes, prolonged residence in body, and a reduction or elimination of protein immunogenicity [173]. PEG contains two equivalent hydroxyl groups, which makes it a potential cross-linking agent for many systems to which it is attached. These hydroxyl groups also provide the possibility for the attachment of bioactives by covalent coupling. Various PEG derivatives such as bromo, amino, carboxymethyl, succinimido succinate, tosylate, mesylate, aldehyde, octadesylamine, monopalmitate, and stearyloxy, or methoxy PEG (MPEG) have been conjugated with dendrimers to form PEGylated dendrimers [174]. MPEG is the derivative of choice in derivatization reactions having one hydroxyl group blocked [175]. The cytotoxicity of cationic melamine dendrimers having surface groups like amine, guanidine, carboxylate, sulphonate or phosphonate has been found to be much higher as compared to anionic or PEGylated dendrimers [25].

In present scenario, surface engineering with different ligands is most frequently used concept in drug delivery, projecting incredible advantages in nanotechnology. Dendrimers like PAMAM, PPI and PLL can be easily decorated with various ligands due to the presence of many surface cationic groups. The attachment of various ligands such as carbohydrates, amino acids, peptides, antibodies, tuftsin, folate, surfactants etc [92,104,139,176–178] not only enhances the targeting potential of conjugates but also enhances their biocompatibility. In reported studies surface of PPI dendrimers were modified with three different ligands including folate, dextran and galactose. As compared to plain PPI, developed conjugates not only exhibited better targeting potential but also drastically decreased hemolytic toxicity than plain PPI [92]. Many available reports clearly support the benefits of dendrimer surface modification with various ligands [99,100,103,137,179,180]. Dendrimer has also been used with various other carriers such as liposomes [6,181], nanoparticles [182–186] and carbon nanotubes (CNTs) [187–189] etc. The developed hybrid systems may have some path-breaking advantages in the field of drug delivery. Various ligands have been used for surface engineering of dendrimers and a detailed report on principle ligands used for engineering and effect of surface engineering on the drug release, toxicity and efficacy of formulation are summerized in Table 5.

Table 5
Summary of surface engineering of dendrimers with various ligands.

Dendrimer	Ligand	Bioactive	Toxicity	IC_{50}	Drug release	Rationale	Reference
PAMAM	Peptide	–		Decreased	–	Novel peptide–dendrimer conjugates as drug carriers for targeting nonsmall cell lung cancer	[231]
PPI	Folate, dextran, galactose	PTX	Decreased	Decreased	Sustained	Cancer targeting potential of some ligand-anchored poly (propylene imine) dendrimers: a comparison	[92]
PPI	Polysorbate 80	DTX	Decreased	Increased	Sustained	The treatment of Glioblastoma xenografts by surfactant conjugated dendritic nanoconjugates	[100]
PPI	Folate	MTX+ATRA	–	Decreased	Sustained	Surface engineered dendrimers for dual drug delivery: a receptor up-regulation and enhanced cancer targeting strategy	[103]
PPI	Dextran	DOX	Decreased	Decreased	Sustained	Dextran conjugated dendritic nanoconstructs as potential vectors for anticancer agent	[99]
PPI	t-BOC-protected glycine-coated dendrimer (DBG), t-BOC-protected phenylal-anine-coated dendrimer (DBPA), mannose-coated dendrimer (M-PPI) and lactose-coated dendrimer (L-PPI)	–	Decreased	Decreased	–	Investigations on the toxicological profile of functionalized 5.0 G PPI dendrimer	[29]
PAMAM	PEG	Fluorouracil (5-FU)	Decreased		Sustained	PEGylated dendritic nanoparticulate carrier of fluorouracil	[232]
PPI	Galactose	Primaquine phosphate	Decreased	–	Sustained	Glycodendrimeric nanoparticulate carriers of primaquine phosphate for liver targeting	[3]
PPI	Mannose	Lamivudine			Sustained	Targeting potential and anti-HIV activity of lamivudine loaded mannosylated PPI dendrimer	[148]
PPI	Tuftsin	Efavirenz	Decreased	Decreased	Sustained	Targeting of efavirenz loaded tuftsin conjugated PPI dendrimers to HIV-infected macrophages *in vitro*	[104]
PPI	Mannose	Rifampicin	Decreased		Sustained	Intracellular macrophage uptake of rifampicin loaded mannosylated dendrimers	[196]
PPI	PEG	Rifampicin	Decreased		Sustained	PEGylated dendritic architecture for development of a prolonged drug delivery system for an antitubercular drug	[215]
PAMAM	Galactose	Chloroquine phosphate	Decreased		Sustained	Glycoconjugated peptide dendrimers-based nanoparticulate system for the delivery of chloroquine phosphate	[90]
PPI	PEG	Famotidine	Decreased	–	Sustained	PEGylated PPI dendritic architectures for sustained delivery of H_2 receptor antagonist	[102]
PAMAM	PEG	–	Decreased	Decreased	–	PEG–PAMAM dendrimers conjugate-mediated efficient intramuscular gene expression	[233]
PAMAM	Iron oxide NPs (IONPs)	Doxorubicin	–	–	Sustained	Novel water-soluble and pH-responsive anticancer drug nanocarriers: Doxorubicin–PAMAM dendrimer conjugates attached to superparamagnetic iron oxide NPs (IONPs)	[234]
PAMAM	PEG	Indomethacin	–	–	Increased	PEGylated thermo-sensitive PAMAM dendritic drug delivery systems	[235]
PAMAM‘	PEG	DOX	Decreased	Increase	Increased	Partly PEGylated PAMAM dendrimer for tumor-selective targeting of doxorubicin, the effects of PEGylation degree and drug conjugation style	[236]
PPI	Sialic acid and mannose	Zidovudine	Decreased	Decreased	Sustained	Sialic acid conjugated-Mannosylated PPI (SMPPI) dual ligand dendritic system for site-specific delivery of anti-HIV drug	[141]
4.0 G PAMAM	Arginine	siRNA				Functionalized dendrimer based delivery of angiotensin type 1 receptor siRNA for preserving cardiac function following infarction	[223]

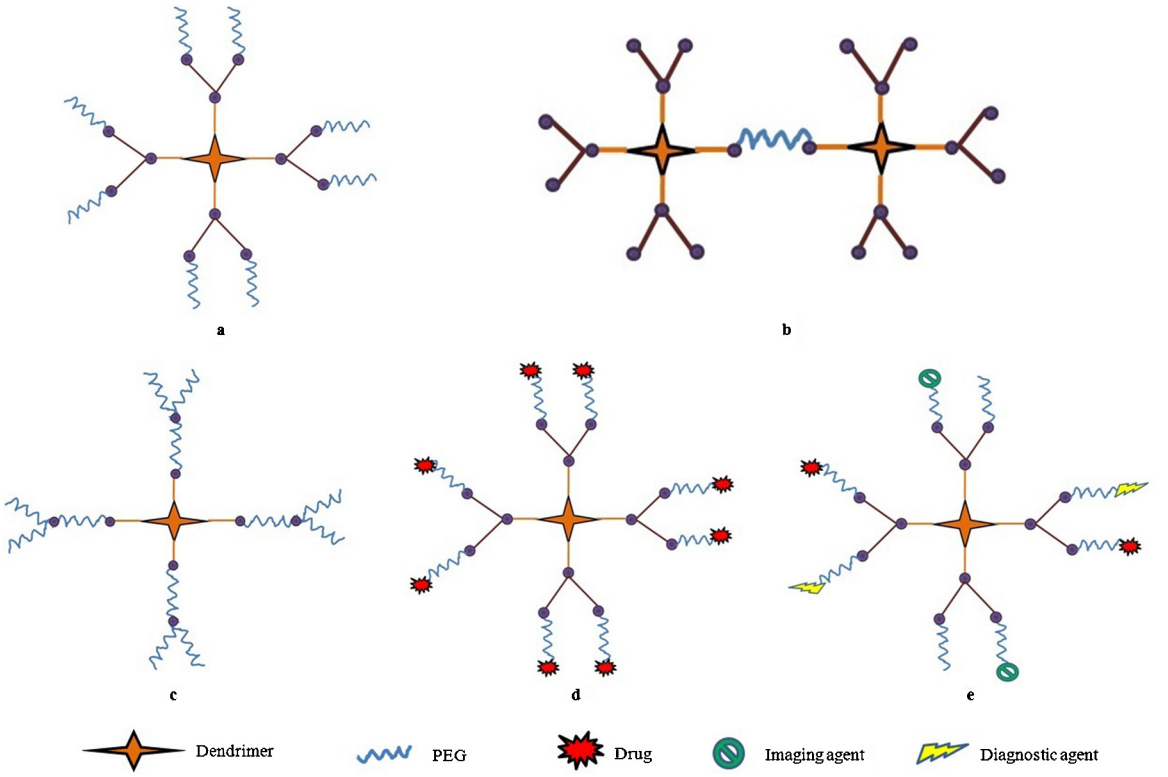

Fig. 15. Approaches to dendrimer PEGylation. (a) Dendrimers with surface PEGylation, (b) PEG cored dendrimers, (c) dendrimers having PEG as branching unit, (d) PEGylated dendrimers conjugated with drug, and (e) PEGylated dendrimers conjugated to multiple ligands for various applications.

10. Dendrimer linked to other nanocarriers

Dendrimers have been linked to different carriers for different pharmaceutical and biomedical purposes (Fig. 16). The conjugates or complexes of dendrimers with different novel drug carriers such as liposomes, CNTs and nanoparticles have resulted in some long term advantages, which have been enlisted in Tables 6–8.

10.1. Dendrimer linked to liposomes

In a study by Jain & Coworkers the encapsulation of MTX in liposomes (acidic drug) was increased through PAMAM dendrimer. Liposmes were prepared by film hydration method using hydrogenated soy phosphatidylcholine, cholesterol and dicetylphosphate in 1.5:1:1 molar ratio, with or without PAMAM dendrimer. Encapsulation of MTX was increased in the presence of dendrimer possibly due to the entrapment of dendrimer by charge interaction that creates pH and solubility gradient across the bilayer and lead to an influx of MTX. The entrapment behavior of drug was proportional to the dendrimer generation. Liposomes containing 4.0 G dendrimer were capable to encapsulate drug approximately two- and four times greater than 3.0 G and 2.0 G dendrimer containing liposomes, respectively, which is ascribed to less solubility of MTX in 3.0 G and 2.0 G dendrimer. Another possibility may be the proportion of these dendrimers attached with the surface phosphate groups that was unable to encapsulate drug due to their open structure [6].

Papagiannaros et al. developed two liposomal formulations, composed of HePC:Egg phosphatidylcholine:Stearylamine 10:10:0.1 (molar ratio) (formulation 1) and Egg phosphatidylcholine:Stearylamine 10:0.1 (molar ratio) (formulation 2) incorporating a doxorubicin (DOX)–PAMAM (3:1 molar ratio) (formulation 3) complex. Lipid bilayers of liposomes composed of hydrogenated soy phospatidyl-choline, cholesterol and dicetylphosphate were attached to PAMAM dendrimers [182]. DOX–PAMAM complex into liposomes displayed low release pattern for DOX as well as high incorporation efficiency, which is essential for the activity of DOX. As compared to formulations 2 and 3, a better *in vitro* cytotoxic activity as well as a delayed release of DOX was observed by the DOX–PAMAM complex attached to formulation 1; tested against various cell lines i.e. lung (DMS114, NCI-H460), colon (HT29, HCT116), breast (MB435, MCF7), prostate (DU145) and CNS (SF268). The two colon cancer cell lines HT29 and HCT116 were found to be somewhat more sensitive in the treatment with formulation 2 than other cell lines suggesting a selective action of DOX.

In final conclusion, the hybrid of dendrimers with liposomes may result in improved therapeutic response due to high drug pay load as well as selective drug delivery to the target site (see Table 6).

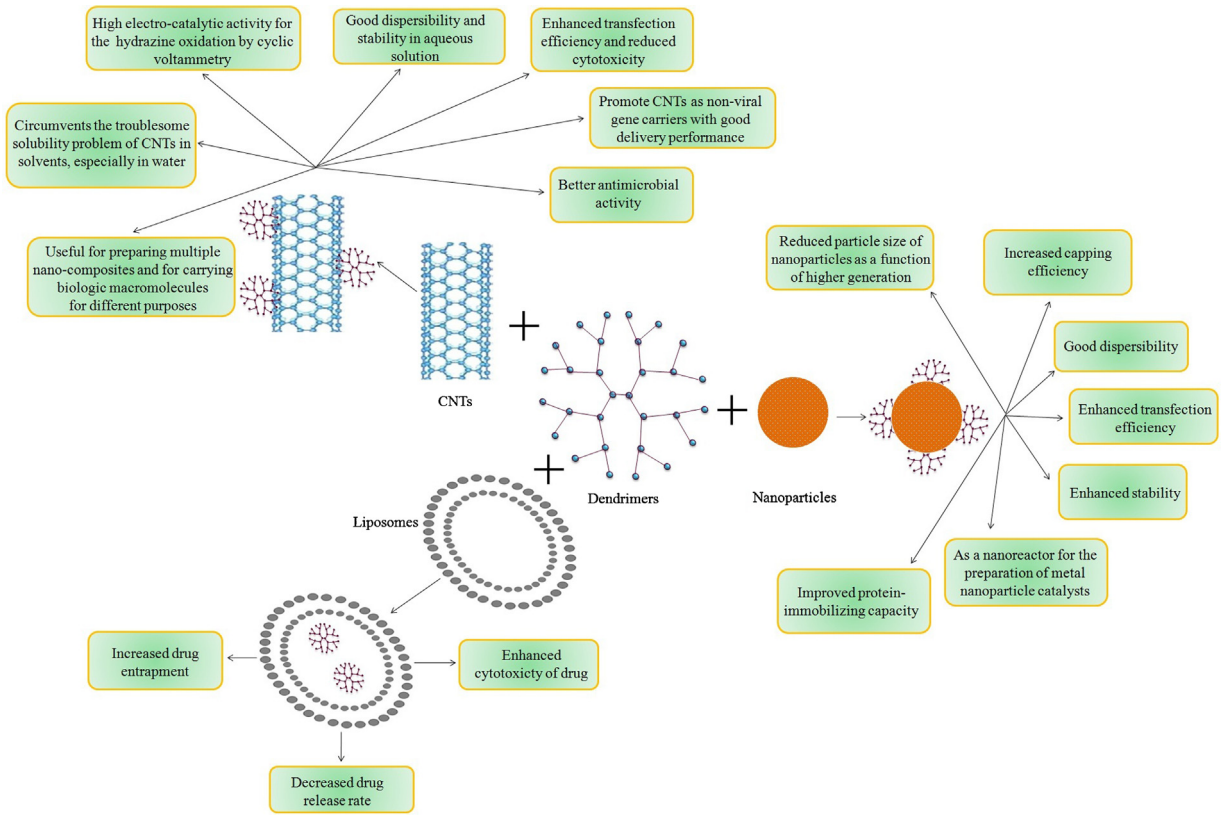

Fig. 16. Dendrimers as co-carriers.

10.2. Dendrimer linked to nanoparticles

An addition of a hydrosilane unit (Si–H) to a double bond is known as hydrosilylation reaction, widely employed in the preparation of silane coupling agents and silicon polymers [190]. Using the principle of this hydrosilylation reaction Li et al. developed platinum nanoparticles wherein carbosilane dendrimer served as capping agent and stabilizer. The surface of Pt nanoparticles was capped by excess hydrosilane, and then stabilized by the dendrimers. The result of X-ray photoelectron spectroscopy confirmed that the Pt surface was directly capped by dendrimers due to the presence of Pt–C bonds because the carbon atom in the Pt–C bonds belongs to the dendrimer. It was concluded that the developed nanoparticles have a good dispersibility under the protection of dendrimers [186].

Nakanishi and Imae evaluated the photocatalytic activity of PAMAM dendrimer-protected TiO_2 nanoparticles

Table 6
Dendrimer linked to liposomes.

Liposomes	Dendrimer	Bioactives	Result	Rationale	References
DSPC:CHOL:PG	Lipidic peptide partial dendrimers	–	Adsorption is inversely proportional to the head size of the partial dendrimer molecules and the extent of adsorption was similar on both positively and negatively charged liposomes	Interaction of cationic partial dendrimers with charged and neutral liposomes	[237]
HePC:EPC:SA	PAMAM	Doxorubicin	Modulatory liposomal controlled release system (MLCRS) seems to be possible for drug delivery and can modulate the release of drugs from dendrimeric liposomal formulations	Cytotoxicity studies of Doxorubicin–PAMAM dendrimer complex attached to liposomes against human cancer cell lines	[182]
HSPC-CH-DCP-GD	PAMAM	Methotrexate	The encapsulation increases with dendrimer generation	Effect of dendrimer on entrapment and release of bioactive from liposomes	[6]

Table 7
Dendrimer linked to nanoparticles.

Nanoparticles	Dendrimer	Result	Rationale	References
Pt NPs	4.0 G of carbosilane dendrimer	Formed Pt NPs have good dispersibility under the protection of dendrimers	Synthesis of Platinum NPs in the hydrosillylation reaction using dendrimer as capping agent and stabilizer	[185]
Pd NPs	Fréchet type 1.0 G dendrimer	Developed NP catalysts capable of catalyzing several reactions	Synthesis of Palladium NP-cored Fréchet type G1-dendrimer (Pd-G1) stabilized by Pd–carbon bonds	[238]
Au NPs	1.0–5.0 G PPI	The resistivity of the films increased exponentially with increasing dendrimer generation	Gold NP/PPI-dendrimer based chemiresistors vapor-sensing properties as a function of the dendrimer size	[239]
Au NPs	4.0 G PAMAM	Oxygen permeability occurs easily for up to three bilayers of PAMAM-Au, with little extra benefit being obtained from using thicker films with more than three bilayers	Oxygen reduction and diffusion in electroactive nanostructured membranes (ENM) using a layer-by-layer dendrimer-gold NP approach	[240]
TiO_2 NPs	4.0 G PAMAM, 4.5 G PAMAM-COONa	Dendrimer acts as a reservoir of photoreacting reagents besides acting as a protector of NPs	Determination of photocatalytic activity of dendrimer-protected TiO_2 NPs in water and comparison with TiO_2 NPs	[183]
Au NPs	4.0 G PAMAM	The PAMAM-Au played an essential role in not only binding the target proteins but also assisting the electron transfer process	Development of electrochemical immunosensor for α-synuclein based on dual signal amplification using PAMAM dendrimer-encapsulated Au and enhanced gold NP labels	[241]
CdS and Au NPs	4.0 G and 7.0 G and PAMAM	Stabilization of polydisperse NPs by both PAMAM–NH_2 and PAMAM/PEG dendrimers	Templating of inorganic NPs by PAMAM/PEG dendrimer–star polymers	[242]
Au NPs	2.0–5.0 G PAMAM	Size of self-assembled cluster decreased with increasing dendrimer generation	Dendrimer-mediated strategy for the self-assembly of gold NPs into structured ensembles	[184]
Au NPs	3.0 G PPI	The dendrimer-protected gold NPs were prepared, size of which can be controlled by molar ratio of dendrimer to gold	Size-controlled synthesis of dendrimer-protected gold NPs by microwave radiation	[243]
Ag NPs	1.5 G PAMAM	PVP and G1.5 PAMAM dendrimer co-mediated Ag NPs were synthesized, reaction rate of which was strongly influenced by the molar ratios of PVP and G1.5 PAMAM dendrimer and the reaction temperature	PVP and 1.5 G PAMAM dendrimer co-mediated synthesis of silver NPs	[186]
Magnetite NPs	5.0 G PAMAM	The dendrimer-modified magnetite NPs were used to carry out magnetic immobilization of BSA. BSA immobilizing efficiency increased with increasing generation from one to five	Synthesis of dendrimer-modified magnetite NPs for protein immobilization	[184]
Pd–Rh bimetallic NPs	4.0 G PAMAM-OH	Resulting dendrimer-encapsulated Pd–Rh bimetallic NPs show a promising catalytic activity in partial hydrogenation of 1,3-cyclooctadiene	Partial hydrogenation of 1,3-cyclooctadiene using dendrimer-encapsulated Pd–Rh bimetallic NPs	[244]
Au NPs	Glycodendrimers, 2.0–5.0 G PPI dendrimers with dense maltose shell	The particle size and size distribution of resulting NPs can be controlled directly as a function of dendrimer's generation with formation of smaller particles at higher dendrimer generations	Oligosaccharide-modified dendrimers for templating gold NPs	[245]

Table 7 (*Continued*)

Nanoparticles	Dendrimer	Result	Rationale	References
Au–Ag alloy NPs	5.0 G PAMAM	Developed dendrimer-entrapped or dendrimer-stabilized Au–Ag alloy NPs should have a promising potential for CT imaging and other biomedical applications	Tunable synthesis and acetylation of dendrimer-entrapped or dendrimer-stabilized gold–silver alloy NPs	[246]
FeS NPs	4.0 G PAMAM	Synthesis and manipulation of FeS NPs onto mesoporous silica microparticles provide versatile platforms for their environmental remediation applications	Synthesis, characterization, and manipulation of dendrimer-stabilized iron sulfide NPs	[247]
Chitosan NPs	1.0 G and 2.0 G PAMAM	Quaternized carboxymethyl chitosan (CM-HTCC)/PAMAM dendrimer NPs displayed higher antibacterial activity against Gram-negative bacteria *Escherichia coli*, whereas they showed much less efficiency against Gram-positive bacteria *Staphylococcus aureus*; compared to quaternized chitosan (HTCC)	Synthesis and characterization of quaternized carboxymethyl chitosan/poly(amidoamine) dendrimer core–shell NPs	[248]
Porous hollow silica NPs	3.0 G PAMAM	3.0 G PAMAM dendrimer-grafted porous hollow silica NPs have been successfully fabricated as photosensitive drug carriers for PDT	PAMAM-grafted porous hollow silica NPs for enhanced intracellular photodynamic therapy	[225]
AuNps	5.0 G PAMAM	Developed gadolium-loaded dendrimer-entrapped gold NPs provided multifunctional nanoplatform for targeted CT/MR dual mode imaging of various biological systems with high accuracy and high sensitivity	Multifunctional dendrimer-entrapped gold NPs for dual mode computed tomography (CT)/magnetic resonance (MR) imaging applications	[249]
AuNps	4.0 G PAMAM	Developed biosensor was successfully applied for the glucose analysis in beverages	Modified gold surfaces by 6-(ferrocenyl)hexanethiol/dendrimer/gold NPs as a platform for the mediated biosensing applications	[213]

in water and compared with necked TiO_2 nanoparticles. 2,4-dichlorophenoxyacetic acid (DPA) was used as a photodegradation reagent for the determination of photocatalyzed degradation mechanism [183]. As compared to TiO_2 nanoparticle without protector, dendrimer-protected TiO_2 nanoparticle was more proficient as a photocatalyst. This was due to the fact that dendrimer works as a reservoir of 2,4-DPA that is trapped in the interior of a dendrimer before oxidation.

The magnetite particles are generally of core–shell type, the biological cells, nucleic acids, and proteins are connected to the magnetite core through an organic or polymeric shell. Pan et al. reported the direct formation of a cascading PAMAM dendrimer on the surface of aminosilane modified magnetite nanoparticles for bovine serum albumin (BSA) immobilization. Thermogravimetric analysis (TGA) has been used for the evaluation of coating formed on the magnetite nanoparticle surface. Authors concluded that the number of surface amines on magnetite nanoparticles was directly proportional to dendrimer generations one through five. It was observed that from generation one to four the amine group becomes double, however in case of generation five the amine groups increased less than twice due to the steric interference between dendrimers [184].

Silver nanoparticles stabilized by polymer can be synthesized by several reported physical and chemical methods [191]. Factors such as selection of the reductant as well as its reducing ability and selection of a suitable protecting agent during the course of reduction of relevant metal salts are important during chemical synthesis approach. Earlier reported method in which polyvinylpyrrolidone (PVP) was used as protecting agent has some disadvantages like the particles prepared by this method were usually irregular in shape and the size would be larger than 100 nm [192]. To overcome these disadvantages, Li et al. developed dendrimer templating synthesis of metallic nanoparticles. Nanoparticles were prepared by reducing silver nitrate with H_2 in the presence of PVP and 1.5 G PAMAM dendrimer as a co-protective agent. These nanoparticles were found to be stable for very long time in appropriately selected solvent systems [186]. The stability as well drug delivery potential is found to be improved by complexation of dendrimers with nanoparticles (see Table 7).

Table 8
Dendrimer linked to carbon nanotubes (CNTs).

CNT	Dendrimer	Result	Rationale	References
MWCNT	G4-PAMAM	The dispersed solution of the hybrid possesses excellent stability in water due to the plenty amino groups on the surface of the CNT–PAMAM	Development of novel functionalized carbon nanotube CNT–PAMAM hybrid by covalently linking PAMAM onto CNTs	[187]
SWCNT	Fluorinated dendrimer	Developed polymeric aggregates had an extremely higher dispersion ability of not only SW-CNT and fullerene but also magnetic NPs into water, compared to that of the corresponding two fluoroalkyl end-capped dimethylacrylamide oligomers	Synthesis and applications of novel fluorinated dendrimer-type copolymers by the use of fluoroalkanoyl peroxide as a key intermediate	[250]
MWCNT	PAMAM	Developed biosensor was used to detect glutamate as substrate with high sensitivity, low detection limit, fast response and nearly free of interference	An enhanced biosensor for glutamate based on self-assembled carbon nanotubes and dendrimer-encapsulated platinum nanobiocomposites-doped polypyrrole film	[188]
SWCNT	DAB-dendrimer-$[NH_2]_8$	Developed nanocluster molecules possess uniform size and well-defined molecular structure; they are fundamentally different from cobalt colloids or other NPs formed from decomposition of a salt precursor	Dendrimer based cobalt (Co_{32}) nanocluster: synthesis and application in diameter-controlled growth of single-walled carbon nanotubes	[251]
MWCNT	PAMAM	Prepared Pd/4.0 G-NH_2/MWNTs exhibited high electro-catalytic activity towards hydrazine oxidation, suggesting that the nanocomposites have good potential application in fuel cells	Dendrimer-encapsulated Pd NPs anchored on carbon nanotubes for electro-catalytic hydrazine oxidation	[181]
MWCNT	PAMAM	The MWCNT–PAMAM hybrid improved the transfection efficiency and simultaneously decreased the cellular toxicity	Improved gene intracellular transporting (GFP) gene transfection mediated by PAMAM dendrimer-functionalized MWCNTs with high biocompatibility	[189]
MWCNT	PPI	The antimicrobial activity of MWCNTs-APPI and MWCNTs-APPI-AgNPs against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* was achieved. The order of activity in terms of percentage of kill was MWCNTs-APPI-AgNPs > MWCNTS-APPI > MWCNTS-COOH	Effective functionalization of MWCNTs with amphiphilic PPI dendrimer carrying silver NPs for better dispersability and antimicrobial activity	[252]
MWCNT	2.0 G and 3.0 G PPI	MWCNT-APPI (3.0 G)-PdNPs was more efficient among the four types of MWCNT nanohybrid catalysts	Synthesis, characterization, and catalytic activity for hybrids of MWCNT and amphiphilic PPI dendrimer immobilized with silver and palladium NP	[253]

10.3. Dendrimer linked to CNTs

Due to excellent electrical functions of CNT–PAMAM, it has been evaluated as a potential carrier for biosensor design by Zeng et al. A bi-enzymatic CNT–PAMAM was prepared by immobilizing glucose oxidase (GOx) and horseradish peroxide (HRP) on the CNTP-AMAM matrix. In water, CNT–PAMAM possesses good dispersability with plenty of functional groups on the surface. The presence of amino groups on the CNT–PAMAM surface allows it to be functionalized by different generations of PAMAM. These developed hybrids were found to be capable of carrying biologic macromolecules for different purposes. Thus the functionalized CNTs are very effective when they are used for constructing the platform of biosensors [187]. In another study an enhanced biosensor for glutamate based on self-assembled CNTs and dendrimer-encapsulated platinum nanobiocomposites-doped polypyrrole film was developed. The biosensor was claimed to show performance characteristics with rapid response, high sensitivity, low level of interference, low detection limit and excellent reproducibility and stability [188].

A facile method for the advancement of amine-terminated PAMAM dendrimer (4.0 G-NH_2)-encapsulated palladium nanocatalysts with CNTs as a support (Pd/4.0 G-NH_2/MWNTs) was reported by Shen et al. Developed system characterized by UV–vis spectroscopy, X-ray diffraction (XRD), TEM confirmed them to be in nanometric range. High electro-catalytic activity towards hydrazine oxidation of nanocomposites proved their potential application in fuel cells [181]. Using covalent linkage approach, Qin et al. successfully prepared PAMAM dendrimer-functionalized MWCNTs for green fluorescence protein (GFP) gene delivery into HeLa cells. As compared to mixed acid-treated MWCNTs (MWCNT-COOH) and pure PAMAM dendrimers, the transfection efficiency of MWCNT–PAMAM hybrid was improved 2.4 and 0.9 times, respectively. Based on this, authors concluded that the transfection efficiency of MWCNTs could be improved through modification with PAMAM dendrimers

and this hybrid could provide a good option for delivering biomolecules into mammalian cells [189].

All these studies suggest that the hybrids of two most recent nanocarriers i.e. dendrimers and CNTs can provide a new vista in the field of medicine (see Table 8).

11. Biofate of dendrimers

Very few reports are available on the fate of dendrimer in the biological system. Jain & coworkers studied the biofate of dendrimers by investigating the biodistribution of plain as well as glycosylated (mannosylated and lactosylated) PPI dendrimers labeled with radioactive technetium (sodium pertechnetate; $^{99m}TcO_4^-$) in female Balb/c mice. In this study the authors assessed the various pharmacokinetic parameters such as clearance, plasma drug concentration profile, distribution and uptake of these dendrimers. In the result it was found that both surface modified and unmodified PPI dendrimers were excreted through kidney and were preferentially taken up and retained by liver although the retention was remarkably high for glycosylated dendrimers. It was observed that the dendrimers showed the clearance pattern that was concentration-dependent and biphasic. Also the clearance of surface modified dendrimers was faster than the plain dendrimers [87].

Kaminskas et al. examined the factors affecting the *in vivo* deposition of dendrimers and found that size and surface charge played a crucial role after parenteral, non-parenteral and interstitial administration. However lipophilicity and hydrophilicity are yet another aspects, which affect *in vivo* deposition of dendrimers after parenteral and interstitial administration, respectively. Size of the dendrimer may influence the clearance mechanisms of dendrimers, for example small uncharged dendrimers (<25 kDa) cleared rapidly from blood via urinary excretion however in case of large uncharged dendrimers, clearance mechanisms typically shift to non-renal clearance processes [40].

Dendrimers have been designed to be administered by different routes, which result in different rates of drug absorption. For example, after oral absorption the order of increasing permeability of PAMAM dendrimer for Caco-2 cells was cationic > anionic > uncharged or PEGylated; however in case of percutaneous absorption the order was cationic > uncharged > anionic [40]. From the above mentioned account it may be concluded that in view of grossly inadequate studies available on the biofate of dendrimers there remains need for more intense research on the interaction of dendrimers and biological environment so as to have clear picture on the exploration of dendrimers for clinical applications.

12. Regulatory considerations

The objective of regulatory affairs is to protect public health. It is mandatory to control the safety and efficacy of products, which are intended to be used clinically such as pharmaceuticals, medical devices, veterinary medicines, agrochemicals and cosmetics. Regulatory affairs are particularly important in case of health-care industries like pharmaceuticals, foods, *in vitro* diagnostics, biologicals, nutritional products, cosmetics and medical devices. The continuously emerging new concepts in the field of drug delivery like nanocarriers (nanoparticles, dendrimers, carbon nano tubes etc.), diagnostic agents, bioactives etc. have increased the importance of regulations for new pharmaceutical products. It would be profitable for dendrimeric formulation based market, if dendrimers attains the status of generally referred as safe (GRAS). Recently, Starpharma has started the phase 3 clinical trials of VivaGel® (SPL7013), which is a dendrimer based formulation for treatment of bacterial vaginosis. The advantages of VivaGel® over conventional antibiotics for treatment of bacterial vaginosis are depicted in Fig. 17. It is a major milestone in the field of dendrimer-mediated therapeutics and will possibly lead to new vistas in the field of medicine [193].

13. Conclusion and future prognosis

Accurate selection of the delivery system is a key factor in the arena of drug delivery. The application of nanotechnology in drug delivery has witnessed exponential growth in the past few years. It is a technological breakthrough, which is being realized very fast, from concept to reality. In the field of nanotechnology, dendrimers emerge as a potential macromolecule for biomedical, pharmaceutical and biopharmaceutical applications in the 21st century. Dendrimers popularity has increased almost exponentially, for example the number of manuscripts published on dendrimers each year has increased from approximately 40 in 1999 to over 520 in 2012 (PubMed database) (Fig. 18) [194]. Dendrimers establish an electrifying opportunity for scientists to fabricate macromolecular structures with a specifically tailored function. In particular, the advantage of dendrimers over other macromolecular constructs lies in fine tuning the potential to generate highly monodisperse systems with good control over final size and surface functionality. This precisely controlled architecture allows modification of dendrimers as per the requirements, which makes these compounds ideal (possibly?) carrier in the field of medicine. Additionally, the irritating behavior of polycationic dendrimers toward biomembranes creates transient nanoholes, which provide another option in the exchange of payload across the biomembrane. The tailor-made surface of dendrimers provides opportunities for designing and tuning properties that are not possible with other types of nanocarriers and have shown that they may have a bright future as a new generation of drug delivery. Menjoge et al. investigated the transfer of PAMAM dendrimers across the human placenta and observed that drug conjugated dendrimers were inable to cross human placenta in *ex vivo* studies suggesting utility of dendrimers for controlled delivery of bioactives in pregnant women. But it would be premature to stack any claim in absence of conclusive proof of dendrimer safety and their deposition in human body [195].

Finally, we can conclude that dendrimers have shown significant potential as a versatile delivery system for drugs, gene, DNA, oligonucleotides, proteins, peptides; as well as diagnostic agent, solubilizing agent etc. However the story of dendrimer from bench of chemist to formulation

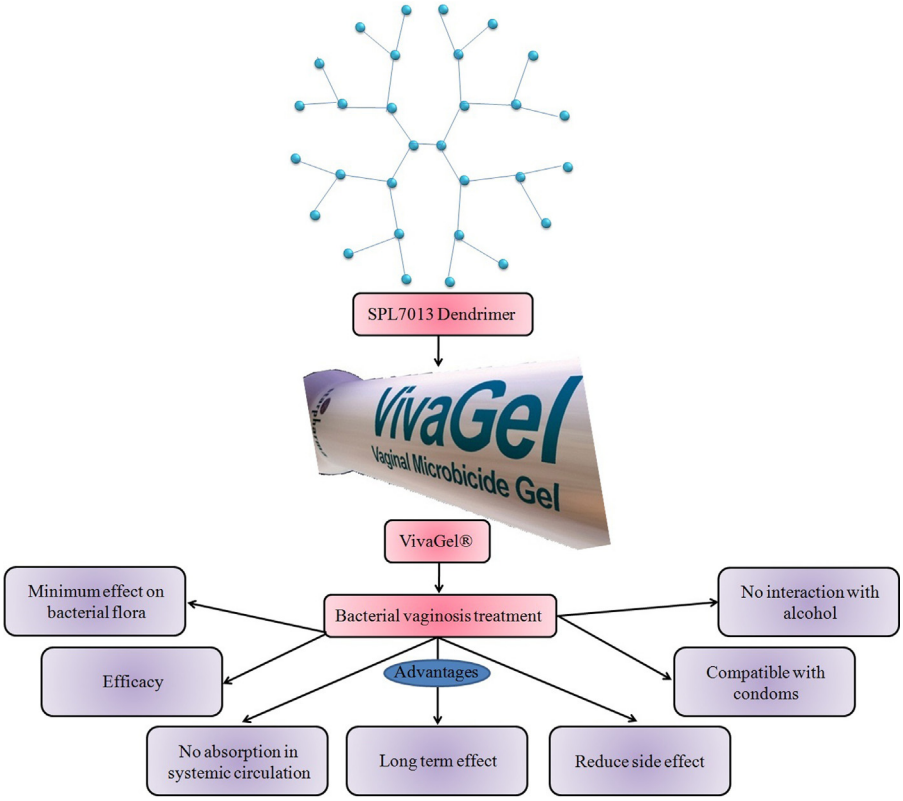

Fig. 17. Advantages of VivaGel® over conventional bacterial vaginosis.

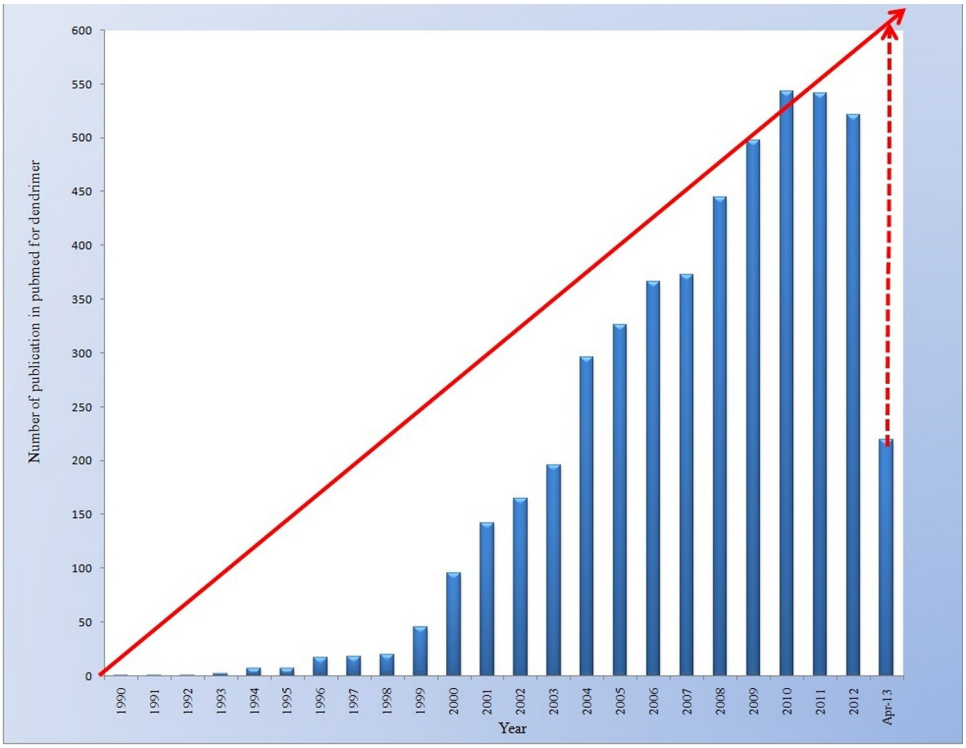

Fig. 18. Yearwise number of dendrimer publications [Pubmed].

desk is still far from complete. Lot of vistas remains to be unfolded. Still dendrimer applications to drug delivery are in infancy and scientists are exploring different aspects of dendrimers as drug delivery vehicle. Interdisciplinary vision on dendrimer hybrids with other nano delivery systems may emerge as a rewarding strategy. However the toxicity of the constituents of hybrid nanosystems *vis a vis* the hybrid itself will have to be explored and established, if not ruled out.

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