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Dendrimers: Nanopharmaceuticals for Drug Delivery

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ABSTRACT

Dendrimers are new class of polymeric materials. It is generally described as a macromolecule, which is characterized by its extensively branched 3D structure that provides a high degree of surface functionality and versatility. Dendrimers are highly branched, globular macromolecules with many arms emanating from a central core. The unique properties associated with these dendrimers such as uniform size, high degree of branching, water solubility, multivalency, well-defined molecular weight and available internal cavities make them attractive for biological and drug-delivery applications. Present review will have main focus on advantages, different synthesis strategies of dendrimers, types of dendrimers and recent studies on important applications of dendrimers.

Keywords: Dendrimers, Drug Delivery, Imaging Agents, Biocompatibility

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INTRODUCTION

About forty percent of newly developed drugs are rejected by the pharmaceutical industry and will never benefit a patient because of poor bioavailability due to low water solubility and/or cell membrane permeability. New delivery technologies could help to overcome this challenge. Nanostructures with uniform and well-defined particle size and shape are of eminent interest in biomedical applications because of their ability to cross cell membranes and to reduce the risk of premature clearance from the body. The high level of control over the dendritic architecture (size, branching density, surface functionality) makes dendrimers ideal carriers in these applications. These have unique characteristics including monodispersity and modifiable surface functionality, along with highly defined size and structure constituted of three distinct domains: (i) a central core which is either a single atom or an atomic group 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 in the exterior of the macromolecule, which play a key role in the properties. This makes these polymers attractive candidates as carriers in drug delivery applications [1]. Many commercial small drug molecules with anticancer, anti-inflammatory, and antimicrobial activity have been successfully associated with dendrimers Drug delivery can be achieved by coupling a drug to polymer through one of two approaches. Hydrophobic drugs can be complexed within the hydrophobic dendrimer interior to make them water-soluble or drugs can be covalently coupled onto the surface of the dendrimers [2].The loading ability of drug molecules and other bioactive agents can be altered by varying dendrimer generations, the water solubility, biodistribution, circulation time in blood and therapeutic efficiency of drugs in dendrimer-based formulations can be tuned by varying dendrimer surface components, the release of drugs from dendrimer scaffolds can be controlled by using different degradable linkers between dendrimers and drugs, and the specific accumulation of the dendrimer-based therapeutics can be achieved by further modifying the dendrimers with targeting moieties [3]. These properties together prove dendrimer perfect candidate in the design of new drug delivery systems.

Dendrimers offer several featured advantages as drug carrier candidates.

These advantages include

- Well-defined globular structure, predictable molecule weight and monodispersity of dendrimers ensure reproductive pharmacokinetics [4,5].
- Controllable size (generation-dependent) of dendrimers satisfies various biomedical purposes [7-11].
- High penetration abilities of dendrimers through the cell membrane cause increased cellular uptake level of the drugs complexed or conjugated to them [6,12,13].
- The lack of immunogenicity of dendrimers makes them much safer choices than synthesized peptide carriers and natural proteincarriers [14].
- Enhanced penetration and retention (EPR) effect of dendrimers offers preferential uptake of the materials by cancer tissues [15].
- Well-established methodologies proposed to construct nanodevices with various functional moieties based on dendrimers provide miscellaneous biomedical applications of these promising materials, such as cancer targeting therapy, magnetic response imaging, photodynamic therapy, neutron capture therapy [16-19].
- Perfectly programmed release of drugs or other bioactive agents from dendrimers leads to reduced toxicity, increased bioavailability and simplified dosing schedule [20-22]. Generally, the size, shape, and surface properties of the polymeric carriers greatly influence the pharmacodynamic (PD) and pharmacokinetic (PK) behaviors of drugs encapsulated in/complexed to/conjugated to the carrier [23].

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Dendrimer synthesis

The three traditional macromolecular architectural classes (i.e., linear, cross-linked, and branched) are widely recognized to generate rather polydisperse products of different molecular weights. In contrast, the synthesis of dendrimers offers the opportunity to generate monodisperse, structure-controlled macromolecular architectures similar to those observed in biological systems [24-26]. Commercial quantities of controlled structures with polydispersities of Mw/ Mn~1.0005 have been routinely synthesized using traditional organic reagents and monomers such as ethylenediamine and alkyl acrylates. These nontraditional structures are referred to as dendrons or dendrimers. Since 1979, two major strategies have evolved for dendrimer synthesis. The first, introduced by Tomalia, was the bdivergent methodQ in which growth of a dendron originates from a core site. This approach involves assembling monomeric modules in a radial, branch-upon-branch motif according to certain dendritic rules and principles [27]. The second method, pioneered by Hawker and Fre´chet, follows a bconvergent growth process [28]. It proceeds from what will become the dendrimer surface inward to a reactive focal point, leading to the formation of a single reactive dendron. To obtain a dendrimers several dendrons are reacted with a multifunctional core to yield such a product. Using these two key synthetic strategies, over one hundred compositionally different dendrimer families have been synthesized and over 1000 differentiated chemical surface modifications have been reported [29-36]. Most divergent dendrimer syntheses require excess monomer loading and lengthy chromatographic separations, particularly at higher generations. On the other hand, convergent synthesis strategies are generally limited to the construction of only lower generation dendrimers due to the nanoscale steric issues that are encountered when attaching the dendrons to the molecular level core [37]. Simplifying the synthetic preparation of dendrimers thus has been a major challenge and an obstacle for the commercial utilization of these unique structures in industrial areas that require large quantities ofinexpensive materials.

Very recently two new breakthrough approaches in dendrimer synthesis have been reported. The first strategy, coined dlegoT chemistry, utilizes highly functionalized cores and branched monomers to create phosphorus dendrimers. Several variations of the general synthetic scheme, which are interchangeable, have been developed, allowing multiplication of the number of terminal surface groups from 48 to 250 in one step, for example. These dendrimers require just one step per generation performed in a minimum volume of solvent, allow facile purification (i.e., simple washings), and produce environmentally benign byproducts such as water and nitrogen [38,39]. The second approach is based on dclickT chemistry, i.e., the near perfect reliability of the Cu (I)-catalyzed synthesis of 1, 2, 3-triazoles from azides and alkynes to produce dendrimers with various surface groups in high purity and excellent yield. All generation 2 and some generation 3 dendrimers were isolated directly as pure solids without chromatographic separations, and the only major byproduct formed in the reaction is sodium chloride [40]. As early as 1984, PAMAM dendrimers were the first complete dendrimer family (G=0–7) to be synthesized and characterized, followed by commercialization in 1990 [41]. They are synthesized by the divergent method, involving a two-step iterative reaction sequence that produces concentric shells of branch cells (generations) around a central initiator core. This PAMAM core–shell architecture grows linearly in diameter as a function of added generations, while the surface groups amplify exponentially at each generation. As a consequence, tethered congestion occurs as a function of core and branch cell multiplicities to produce geometrically closed nanostructures that exhibit guest–host container properties as discussed later. For the PAMAM dendrimers family initiated from an ethylenediamine core with a branch cell multiplicity of two, the expected mass values double, approximately, from generation to generation. These values have been verified by electrospray ionization (ESI) and matrix-assisted laser desorption/ ionization (MALDI) mass spectroscopy methods. The diameters of these spheroids increase systematically at a rate of approximately 1 nm per generation. There is, of course, the possibility of errors or defects in these divergent dendrimer constructions. However, their monodispersity is remarkable, with polydispersity values (Mw/Mn) in the range from 1.000002 to 1.005 within this series, as verified by narrow bands in gel electrophoresis and ESI/MALDITOF mass spectrometry [42]. At least 100 other dendrimer families, possessing compositionally different interiors (i.e., carbon, nitrogen, silicon, sulfur, phosphorus or metals) and multiplicity values have been synthesized and characterized to date [43].

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Dendrimers in drug delivery

In addition to DNA, dendrimers have been utilized to carry a variety of small molecule pharmaceuticals. Encapsulation of the well-known anticancer drug cisplatin within PAMAM dendrimers gave conjugates that exhibited slower release, higher accumulation in solid tumors, and lower toxicity compared to free cisplatin. Similarly, the encapsulation of silver salts within PAMAM dendrimers produced conjugates exhibiting slow silver release rates and antimicrobial activity against various Gram positive bacteria [44,45]. In another study, PAMAM dendrimers with 4, 8, and 16 terminal ester groups were converted to hydroxy-terminated molecules to reduce their potential cytotoxicity. These dendrimers were able to encapsulate small acidic molecules such as benzoic acid and 2,6-dibromo-4-nitrophenol in 1:1 and 2:1 (drug : dendrimer) ratios but did not form a complex with the non-acidic drug tioconazole. Presumably, the guest molecules were retained within the dendritic branching clefts by hydrogen bonding with interior protonated amide groups. Therefore, the inclusion complexes were observed to separate after deprotonation of these amide groups at pHb7 [47].

Dendrimers in oral drug delivery

Oral drug-delivery system has been the dominant route for many years because of its significant advantages. It is by far the most convenient administration route with good patient compliance, especially in the patient's opinions. Along with these benefits, there are also some defects of oral delivery route like low solubility in aqueous solutions and low penetration across intestinal membranes [47]. D'Emanuele and his research group [48] investigated effect of dendrimer generation and conjugation on the cytotoxicity, permeation and transport mechanism of PAMAM dendrimer and surface-modified cationic PAMAM dendrimer using monolayers of the human colon adenocarcinoma cell line, Caco-2. As increase in the concentration and generation, there was increase in the cytotoxicity and permeation of dendrimers. While reduction in cytotoxicity observed by conjugation with lauryl chloride. Modified dendrimers also reduced transepithelial electrical resistance (TEER) and significantly increased the apparent permeability coefficient (Papp). In another study of transepithelial permeability of naproxen, a low solubility model drug was investigated [49]. The stability of these G0 PAMAM conjugates in 50% liver homogenate was compared to that in 80% human plasma showed the lactate ester linker gave prodrug of high stability in plasma with slow hydrolysis in liver homogenate; such conjugates may have potential in controlled release systems, while using diethylene glycol as a linker gives conjugate that showed high chemical stability, but readily released drug in plasma and liver homogenate. So, these conjugations demonstrate potential as nanocarriers for the enhancement of oral bioavailability.

Dendrimers as a carrier for drug delivery

Dendrimers have narrow polydispersity; nanometer size range of dendrimers can allow easier passage across biological barriers. All these properties make dendrimers suitable as host either binding guest molecules in the interior of dendrimers or on the periphery of the dendrimers.

Dendrimers in transdermal drug delivery

In recent era, dendrimers have found applications in transdermal Drug-delivery systems. Generally, bioactive drugs have hydrophobic moieties in their structure, resulting in low water solubility that inhibits efficient delivery into cells. Dendrimers designed to be highly water-soluble and biocompatible have been shown to be able to improve drug properties such as solubility and plasma circulation time via transdermal formulations and to deliver drugs efficiently. Nonsteroidal anti-inflammatory drugs (NSAIDs) are very effective in the treatment of acute and chronic rheumatoid and osteoarthritis, however, clinical use of NSAIDs is often limited by adverse events such as gastrointestinal side effects (dyspepsia, gastrointestinal bleeding), renal side effects when given orally. Transdermal drug delivery overcome these adverse effects and also maintains therapeutic blood level for longer period of time. Transdermal delivery suffers poor rates of transcutaneous delivery due to barrier function of the

skin. PAMAM dendrimer complex with NSAIDs (e.g. Ketoprofen, Diflunisal) could be improving the drug permeation through the skin as penetration enhancers [50]. The model drugs Ketoprofen and Diflunisal were conjugated with G5 PAMAM dendrimer and investigated for different studies. In vitro permeation studies on excised rat skin showed 3.4 times higher permeation of Ketoprofen from Ketoprofen–dendrimer complex than that from 2mg/mL Ketoprofen suspended in normal saline. Similarly, a 3.2 times higher permeated amount was observed with Diflunisal–dendrimer complex. Anti-nociception effect of drugs was studied on mice, results showed that Ketoprofen–dendrimer complex reducing writhing activity during the period of 1–8 h after Transdermal administration, while pure Ketoprofen suspension at the equivalent dose of Ketoprofen significantly decreased number of writhing between 4 and 6 h after drug was transdermally given.

Dendrimers in ocular drug delivery

The topical application of active drugs to the eye is the most prescribed route of administration for the treatment of various ocular disorders. It is generally agreed that the intraocular bioavailability of topically applied drugs is extremely poor. These results mainly due to drainage of the excess fluid via nasolacrimal duct and elimination of the solution by tear turnover. Several research advances have been made in ocular drug-delivery systems by using specialized delivery systems such as polymers, liposomes, or dendrimers to overcome some of these disadvantages. Ideal ocular drug-delivery systems should be nonirritating, sterile, isotonic, biocompatible, does not run out from the eye and biodegradable [51]. Dendrimers provide unique solutions to complex delivery problems for ocular drug delivery. Recent research efforts for improving residence time of pilocarpine in the eye was increased by usingPAMAMdendrimers with carboxylic or hydroxyl surface groups. These surface-modified dendrimers were predicted to enhance pilocarpine bioavailability [52].

Dendrimers in pulmonary drug delivery

Dendrimers have been reported for pulmonary drug delivery also [53]. During one study, efficacy of PAMAM dendrimers in enhancing pulmonary absorption of Enoxaparin was studied by measuring plasma anti-factor Xa activity, and by observing prevention efficacy of deep vein thrombosis in a rodent model. G2 and G3 generation positively charged PAMAM dendrimers increased the relative bioavailability of Enoxaparin by 40%, while G2.5 PAMAM, a half generation dendrimers, containing negatively charged carboxylic groups had no effect. Formulations did not adversely affect mucociliary transport rate or produce extensive damage to the lungs. So the positively charged dendrimers are suitable carrier for Enoxaparin pulmonary delivery.

Dendrimers in targeted drug delivery

Nowadays general cancer chemotherapeutics are less effective in their ability to cure tumors because of the nonselective action of these highly potent drugs, resulting in dose limiting side effects. The application of drug carrier systems for targeting tumor cells has gained credence as an alternative approach for treating cancer and offers both increased therapeutic index and decreased drug resistance. An effective targeting drug-delivery system requires a base that is uniform and able to couple multiple components such as targeting molecule, drug and cancer imaging agent [54]. Dendrimers have ideal properties which are useful in targeted drug-delivery system. One of the most effective cell-specific targeting agents delivered by dendrimers is folic acid. Membrane associated high-affinity folate receptors are folate-binding proteins that are over expressed on the surface of different types of cancer cells (e.g. ovarian). PAMAM dendrimers conjugated with the folic acid and fluorescein isothiocyanate for targeting the tumor cells and imaging respectively. Further these two molecules are linked with complementary oligonucleotides. DNA-assembled nanoclusters were evaluated in vitro which helps in detecting tumor cell-specific binding and internalization. These DNA-assembled dendrimer conjugates may allow the combination of different drugs with different targeting and imaging agents so it is easy to develop combinatorial therapeutics [55].

Dendrimers for controlled release drug delivery

Fréchet and co-workers have prepared polyaryl ether dendrimers containing dual functionality on the surface. One is used to attach polyethylene glycol (PEG) units on the surface to improve water solubility and the other one is utilized to attach hydrophobic drug molecules. They have also synthesized a series of dendritic unimolecular micelles with a hydrophobic polyether core surrounded by a hydrophilic PEG shell for drug encapsulation. A third-generation micelle with indomethacin entrapped as model drug gives slow and sustained in vitro release, as compared to cellulose membrane control [56].

Dendrimers in gene delivery

Dendrimer-based transfection agents have become routine tools for many molecular and cell biologists but therapeutic delivery of nucleic acids remains a challenge. Because of their immunogenicity, dendrimers are extensively used as non-viral vector for gene delivery [57, 58]. Besides of that some research recently indicated that dendrimer based gene delivery system also have significant potential in clinical trials. Kukowska-Latallo et al. reported that intravenous administration of G9 PAMAM dendrimer-complexed pCF1CAT plasmid could result in high level of gene expression in the lung tissues of rats. It enhances the transfection efficiency and expression pattern of dendrimers [59]. Amphiphilic dendrimers having a rigid diphenylethyne core featured a variety of geometries and substitution patterns, all of which showed high transfection activity. The hydrophobic parameters influenced the DNA binding and transport more strongly than anticipated, exhibiting lower toxicity.

Dendrimers as imaging agents

The first in vivo diagnostic imaging applications using dendrimer-based MRI contrast agents were demonstrated in the early 1990s by Lauterbur and colleagues [60]. In comparison with the commercially available small-molecule agent (Magnevist, Schering, AG), the dendrimer-based reagents exhibited blood pool properties and extraordinary relaxivity values when chelated gadolinium groups (Magnevist®). These generations dependent, dramatic enhancements of MRI contrast properties were some of the first examples of a ‘dendritic effect’ [61].

Biocompatibility of dendrimers

A major concern when introducing a new class of nanoparticles for medical applications is directed towards the biocompatibility of these particles. In order to be usable in drug delivery applications, dendrimers have to be non-toxic and non-immunogenic. Most of these studies are very recent, and therefore, the cytotoxicity of dendrimers has been primarily evaluated in vitro; however, a few in vivo studies have been published [62-66]. As observed for other cationic macromolecules including liposomes and micelles, dendrimers with positively charged surface groups are prone to destabilize cell membranes and cause cell lysis. For example, in vitro cytotoxicity, IC50 measurements (i.e., the concentration where 50% of cell lysis is observed) for poly(amidoamine) PAMAM dendrimers with amino surface revealed significant cytotoxicity on human intestinal adenocarcinoma, Caco-2 cells [67,68].

Furthermore, the cytotoxicity was found to be generation dependent, with higher generation dendrimers being the most toxic [69]. A similar generation dependency of amino-terminated PAMAM dendrimers was observed for the haemolytic effect, studied on a solution of blood cells [70]. However, some recent studies have shown that amino-terminated PAMAM dendrimers exhibit lower toxicity than more flexible linear polymers carrying amine groups, perhaps due to lower adherence of the rigid globular dendrimers to cellular surfaces. The degree of substitution as well as the type of amine functionality is important, with primary amines being more toxic than secondary or tertiary amines. Amino-terminated poly(propylene imine)-PPI dendrimers behave similarly as PAMAM dendrimers with regard to cytotoxicity and haemolytic effects, including the generation-dependent increase both effects [71].

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CONCLUSIONS

The culmination of various advances in dendrimer-based delivery systems along with fundamental work performed over the last couple decades has led to the founding of several start-up companies and a large number of patents focused, at least in part, in the development of dendrimer technologies [62-65]. Although dendrimer drug delivery is in its infancy, it offers several attractive features. Dendrimers expect to be a potential polymer for biomedical, pharmaceutical and biopharmaceutical fields in the 21st century. Easily controllable features of dendrimers such as their size, shape, branching length, their surface functionality allow to modify the dendrimers as per the requirements, makes these compounds ideal carrier in many of the applications. Still toxicity problems may arise, but they will be resolved by modifying dendrimer structure. Future work is necessary to find out cost-effective synthesis strategies and the relationship between dendrimer and drug molecules for successful commercialization of this technology.

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