

Dendrimers: A novel approach in nano drug delivery

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ABSTRACT Nanotechnology is a multidisciplinary field and has achieved breakthroughs in bioengineering, molecular biology, diagnostics, and therapeutics. Several potential methods are available at present for tissue specific drug delivery at different stages of pathogenesis. Inorganic nanostructures that interface with biological systems have recently attracted widespread interest in biology and medicine. Dendrimers are thought to have potential as novel intravascular probes for both diagnostic (e.g., imaging) and therapeutic purposes (e.g., drug delivery). The successful dendrimers mediated delivery includes the ability to target specific tissues and cell types (primary & secondary targeting) and escape from the uptake by the reticuloendothelial system (RES). It has been found that dendrimers coated with a lung-targeting peptide accumulate in the lungs of mice after i.v. injection. Adding polyethylene glycol to the dendrimers coating slows down opsonization and prevents nonselective accumulation of dendrimers in RES. These results encourage the construction of more complex nanostructures with capabilities such as disease sensing and feedback regulated drug delivery. Therefore, the research on dendrimers is expected to address the formidable challenges encountered in the field of drug targeting, and opens up new vistas in future development of a promisingly active and site-specific delivery system.

Key words: Dendrimer, RES, opsonisation, nanoscopic

Introduction

Traditionally polymer technologies were focused mainly on linear polymers. Currently highly branched polymers have been found in pictures, properties of branched macromolecules are quite different from conventional polymers¹. The unique structural features of dendritic and hyperbranched macromolecules have number of chains whose ends combined with a high degree of branching which leads to a variety of new physical properties when compared with traditional linear polymers^{2,3}. Dendrimers offer a plenty of advantages compared to other architectural forms of polymers that have been used in drug-delivery systems. They have narrow polydispersity, nanometer size range, which can allow easier passage across biological barriers (e.g. small enough to undergo extravasations through vascular endothelial tissues). Host-guest can take place either in the interior (binding groups in the interior of

dendrimers are called endoreceptors) or on the periphery of the dendrimer (groups involved in complexation chemistry on the periphery of the dendrimers are called exoreceptors. (Macromolecular architectures offer unique nanoscopic shapes and surfaces which opens the door for either sub-nanoscope reagents (Le. classical organic types) or nanoscopic reagents (e.g. DNA, IgG antibodies, etc.) to perform reactions. So the structure of these macromolecules has great impact on their applications⁴⁻⁶.

Dendrimers are large and complex molecules having very well-defined chemical structures. They possess three distinguishing architectural components, mainly (a) an initiator core, (b) an interior layer (generations), composed of repeating units, radially attached to the initiator core and (c) exterior (terminal functionality) attached to the outermost interior generation. Dendrimers are generally produced in an iterative sequence of reaction steps, in that each

additional interaction leads to higher generation dendrimer. Each of the new layers creates a new 'generation' along with doubling the number of end groups (or active sites) and approximately double the molecular weight of the previous generation, too⁷⁻¹⁰.

Dendrimers are repeatedly branched molecules. The huge number of papers on dendritic architectures such as dendrimers, dendronized, hyperbranched and brush-polymers has generated a vast variety of inconsistent terms and

definitions making a clear and concise unfolding of this topic highly difficult. The purpose of this article is to provide the vocabulary required for the description of chemical and physical phenomena as well as application aspects associated with the research in the area of dendritic molecules. The pubmed search till the date indicates the trend in dendrimers research, given in fig.1. So if systematically investigated, dendrimers may open new vista in research and therapy¹¹.

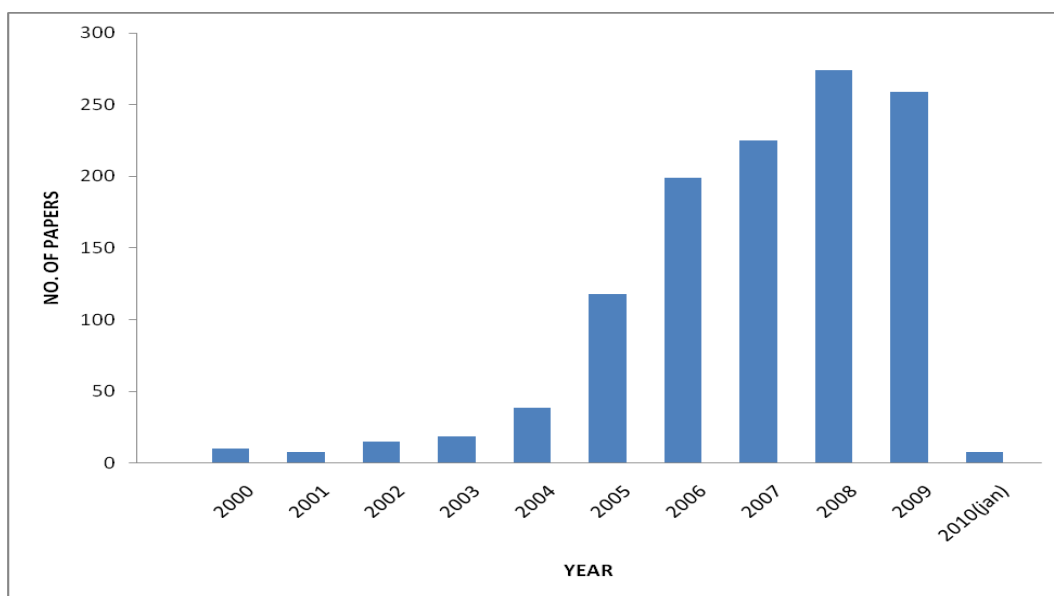


Figure 1: Trends in dendrimers research

The dendritic structure

One of the most interesting topological aspects of dendrimers is the concept of the 'starburst limit'. If one considers that the number of branch ends on a dendrimer increases exponentially as a function of generation, while the surface area of the dendrimer only increases with the square of generation (and the volume with the cube of generation), it is apparent that there will come a point beyond which the dendrimer cannot grow as a consequence of a lack of space. This point is a function of the core multiplicity (n_c), the branching multiplicity (n_b), and the branch length (l_b) as well as of the core and branch volumes and other quantities. The increasing branch density

with generation is also believed to have striking effects on the structures of dendrimers. At high generations, steric crowding of the branches at the surface of a dendritic molecule causes the adoption of a globular conformation. The branch ends may lie either on the surface of the molecule, or throughout the entire structure, possibly determined by factors such as the solvent and the dendrimer constitution. If the former is the case, computer modelling experiments show that the dendrimer will contain cavities and channels. Despite the dynamic nature of these voids, it might be anticipated that the microenvironment they create can be utilised in the entrapment of guest molecules¹²⁻¹⁵.

Construction

Divergent dendrimer growth

The synthetic methodology employed in the early dendrimer synthesis came to be known as the 'divergent' approach. This name comes from the way in which the dendrimer grows outwards from the core, diverging into space. Starting from a reactive core, a generation is grown, and then the new periphery of the molecule is activated for reaction with more monomers. The two steps can be repeated¹⁶. The divergent approach is successful for the production of large quantities of dendrimers since, in each generation adding step, the molar mass of the dendrimer is doubled. Very large dendrimers have been prepared in this way, but incomplete growth steps and side reactions lead to the isolation and characterisation of slightly imperfect samples. Divergently grown dendrimers are virtually impossible to isolate pure from their side products. The synthetic chemist must rely on extremely efficient reactions in order to ensure low polydispersities¹⁷.

Convergent dendrimer growth

The 'convergent' approach was developed as a response to the weaknesses of divergent syntheses. Convergent growth begins at what will end up being the surface of the dendrimer, and works inwards by gradually linking surface units together with more monomers. When the growing wedges are large enough, several are attached to a suitable core to give a complete dendrimer. The advantages of convergent growth over divergent growth stem from the fact that only two simultaneous reactions are required for any generation-adding step. Most importantly, this protocol makes the purification of perfect dendrimers simple¹⁸.

Properties of Dendrimers

Monodispersity

Dendrimers are the class of dendritic polymers that can be constructed with a well-defined molecular structure, i.e being monodisperse, unlike to linear polymers. The monodispersity of dendrimers has

been extensively verified by high performance liquid chromatography (HPLC), size exclusion chromatography (SEC), mass spectrometry (MS), gel electrophoresis and transmission electron microscopy (TEM)¹⁹. As mentioned above, convergent growth process permits the purification process at each step of the synthesis which gives the monodisperse molecules as determined by mass spectrometry. It has been observed that convergently produced dendrimers are probably the most precise synthetic macromolecules that exist today. However, mass spectroscopy has shown that dendrimers produced by divergent method are remarkably monodisperse for earlier generation. Monodispersity offers researchers the possibility to work with a tool for well-defined scalable sizes. This property is useful for applications such as the synthesis of container molecules, use as templates or in electronic applications. The two factors affects the degree of monodispersity includes, (i) dendrimer bridging and (ii) incomplete removal of ethylenediamine at each of the generation sequences. This latter factor at any point in dendrimer growth will function as an initiator core to produce 0.5 generation and subsequent generation dendrimers that leads to polydispersity. Ethylenediamine can be effectively removed from the lower generation dendrimers by simple vacuum distillation at lower temperature and from higher generation dendrimers (Le. $G > 4$) by special techniques like azeotropic (n-butanol) and wiped film distillation²⁰.

Nanoscale size and shape

Based on their dimensional length scaling, narrow size distribution, and other biomimetic properties, dendrimers are often referred an artificial proteins. The shape persistence of dendrimers is very important, as it allows the defined placement of functions not only on the dendrimer surface but also inside the dendritic scaffold. The PAMAM dendrimers of lower generation (G0-G3) with ethylenediamine core have ellipsoidal shapes, whereas the PAMAM dendrimer of higher generation (G4-G10) have roughly spherical shapes. X-ray analysis

on dendrimer aggregates have been revealed that the molecular shape of the lower to higher generations becomes increasingly globular. These fundamental properties have in fact lead to their commercial use for gene therapy, immunodiagnostics and variety of other biological applications^{21,22}.

Polyvalency

Polyvalency of dendrimers provides many interactions as they coordinate to materials. Polyvalency shows the outward presentation of reactive groups on the dendrimer nanostructure exterior. This creates more connections between surfaces and bulk materials for applications such as adhesives, surface coatings, or polymer cross-linking. The product, a topical vaginal microbicide called VivaGel™, prevents infection by HIV and other sexually transmitted diseases during intercourse, takes advantage of dendrimer's polyvalent properties. Depending upon which functional groups are attached to the tips of a dendrimer's branches, these nanostructures can behave like molecular Velcro. Simultaneously, these functional groups can participate in multiple interactions with receptors on biological structures like cell membranes and viruses²³.

Physicochemical properties of dendrimers

Already in the history of the dendrimers it was suggested that nano-sized structure of higher generation dendrimers would serve as synthetic mimics of proteins. The hyperbranched structure of the dendrimer is less compact than a protein, i.e. interior is not packed as efficiently as in typical proteins, and structure of the dendrimer provides a highly multivalent surface, exposing a much higher number of functional groups on the surface compared to proteins of similar molecular size. The use of dendrimers as protein mimics has been encouraged scientists to investigate the physicochemical properties of dendrimers in comparison to proteins shows that dendrimers, similar to protein, can adapt "native" (e.g. tighter) or "denatured" (e.g.

extended) conformations dependent on the polarity, ionic strength and pH of the solvent^{24,25}.

Amino terminated PPI and PAMAM dendrimers (that is the dendrimers having basic surface groups as well as a basic interior) at the low pH region shows the extended conformation due to electrostatic repulsion between the positively charged ammonium groups. At neutral pH back folding occurs which may be due to the hydrogen bonding between the unchanged tertiary amines in the interior and the positively charged surface amines. At pH ≥ 10 conformation has a higher degree of back folding so dendrimer acquires a more spherical (globular) structure, that all because of the weak "inter-dendron" repulsive forces. In comparison with carboxylic acid terminated PPI dendrimers, small angle neutron scattering (SANS) and NMR measurements shows that at pH 2 the dendrimer core has the most extended conformation due to the electrostatic repulsion between the positively charged protonated tertiary amines. At pH 6 slight back folding occurs as a result of attractive Coulomb interactions between the negatively charged surface carboxy-groups and the positively charged tertiary amines in the inner shells of the dendrimer. While at pH 11 the electrostatic repulsion between the negative charged forces the surface groups apart from which gives a more extended conformation with a highly expanded surface area.²⁶

Biocompatibility of dendrimers

In order to utilize dendrimers as biological agents, they have to fulfill certain biological demands. The dendrimer should be:

- non-toxic;
- non-immunogenic (except for vaccines);
- able to cross biobarriers (biopermeable) such as, the intestines, the blood-tissue barriers, cell membranes, etc.;
- able to stay in circulation for the time needed to have a clinical effect;
- able to target to specific structures.

Till today, the cytotoxicity of dendrimers has been primarily studied *in vitro*;

however, a few *in vivo* studies have been reported. Immunogenicity is one of the crucial biological properties of the dendrimers. Studies performed on unmodified amino-terminated PAMAM dendrimers showing no or only weak immunogenicity of the G3-G7 dendrimers. However, later studies showed some immunogenicity of these dendrimers and found that modification of amino-terminated PAMAM dendrimers with polyethylene glycol (PEG) chains reduces immunogenicity and gives longer lifetime in the blood stream in comparison to unmodified dendrimers.

Physical Properties

Dendrimers are of interest for their unusual physical properties. Much work has been carried out in areas such as melt viscosity, glass transition temperature, rheological properties and even on the photo induced electron transfer C60. The most exciting physical property of dendrimers is the variation of their intrinsic viscosities with molecular weight. It is found that, when the generation increases beyond a certain point, the intrinsic viscosity begins to decline, contrary to the behaviour of linear polymers. This effect is believed to be a consequence of the globular shapes of high generation dendrimers leaving them unable to tangle with one another after the manner of linear polymers²².

Types of dendrimers

In recent years, dendrimers with different designed functionalities have become objects of particular academic and practical interest because of their unique super branched architectures, high densities of peripheral functionalities, symmetrical shapes, and monodispersity. Here, some of the dendrimers having different functionalities are briefly described along with their area of utilization.

Liquid crystalline dendrimers

They consist of mesogenic (liq. crystalline) monomers e.g. mesogen functionalized carbosilane dendrimers. Functionalization of end group of carbosilane dendrimers with 36

mesogenic units, attached through a C-5 spacer, leads to liquid crystalline dendrimers that form broad smectic A phase in the temperature range of 17-1300°C. It was shown that such a dendrimer, under UV irradiation can undergo E-Z isomerization of the cinnamoyl groups and [2 + 2] photocycloaddition leading to the formation of a three-dimensional network.

Tecto dendrimers

Tecto-dendrimers are composed of a core dendrimer, which may or may not contain the therapeutic agent, surrounded by dendrimers. The surrounding dendrimers are of several types, each type designed to perform a function necessary to a smart therapeutic nanodevice.

Chiral dendrimers

The chirality in these dendrimers is based upon the construction of constitutionally different but chemically similar branches to chiral core. Chiral, nonracemic dendrimer with well-defined stereochemistry is particularly interesting subclass, with potential applications in asymmetric catalysis and chiral molecular recognition²³.

PAMAMOS dendrimers

Radially layered poly (amidoamine-organosilicon) dendrimers (PAMAMOS) are inverted unimolecular micelles that consist of hydrophilic, nucleophilic polyamidoamine (PAMAM) interiors and hydrophobic organosilicon (OS) exteriors. These dendrimers are exceptionally useful precursors for the preparation of honeycomb-like networks with nanoscopic PAMAM and as domains²⁴.

Hybrid dendrimers

Hybrid dendrimers are combination of dendritic and linear polymers in hybrid block or graft copolymer forms. The small dendrimer segment coupled to multiple reactive chain ends provides an opportunity to use them as surface active agents, compatibilizers or adhesives, e.g. hybrid dendritic linear polymers²⁵.

Peptide dendrimers

Dendrimers having peptides on the surface of the traditional dendrimer framework and dendrimers incorporating amino acids as branching or core units are both defined as 'peptide dendrimers'. Also peptide dendrimers can be defined as macromolecules that contain peptide bonds in their structure. Because of biological and therapeutical relevance of peptide molecules, peptide dendrimers play an important role in diverse areas including cancer, antimicrobials, antiviral, central nervous system, analgesia, asthma, allergy and Ca^{+2} metabolism. On the basis of their ability to be taken up by cells, making peptides very useful for drug delivery. One more interesting application of peptide dendrimers is that can be used as contrast agents for magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), fluorogenic imaging and serodiagnosis²⁶.

Glyco dendrimers

The term 'glycodendrimers' is used to describe dendrimers that incorporate carbohydrates into their structures. Glycodendrimers can be classified as: (i) carbohydrate-coated; (ii) carbohydrate centered; and (iii) fully carbohydrate-based. Glycodendrimers have been used for a variety of biologically relevant applications such as, glycodendrimers with surface carbohydrate units have been used to study the protein-carbohydrate interactions that are in many intercellular recognition events. The accessibility of the sugars is an important consideration for glycodendrimers used effectively to evaluate protein-carbohydrate interactions. Like this, study of protein-carbohydrate interactions, incorporation into analytical devices, formulation of gels, targeting of MRI contrast agents, drugs and gene delivery systems are some of the areas where glycodendrimers are likely to be beneficial²⁷.

PAMAM dendrimer

Perhaps the family of dendrimers most investigated for drug delivery is the polyamidoamine (PAMAM) dendrimer. Many surface modified PAMAM

dendrimers are non-immunogenic, water-soluble and possess terminal-modifiable amine functional groups for binding various targeting or guest molecules. PAMAM dendrimers generally display concentration-dependent toxicity and haemolysis. PAMAM dendrimers are hydrolytically degradable only under harsh conditions because of their amide backbones, and hydrolysis proceeds slowly at physiological temperatures. The internal cavities of PAMAM dendrimers can host metals or guest molecules because of the unique functional architecture, which contains tertiary amines and amide linkages. PAMAM dendrimers are generally prepared by divergent method and product upto generation 10 (G10) have been obtained. PAMAM dendrimers are the most extensively reported moiety for almost all existing applications of dendrimers.

Applications

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 dendrimers²².

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, Oiflunisal) could be improving the drug permeation through the skin as penetration enhancers²⁴.

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. For example the results of propranolol-dendrimer showed that it was able to bypass the P-gp efflux transporter¹⁸.

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. 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 using PAMAM dendrimers with carboxylic or hydroxyl surface groups. These surface-modified dendrimers were

predicted to enhance pilocarpine bioavailability²³.

Dendrimers in pulmonary drug delivery

Dendrimers have been reported for pulmonary drug delivery also. 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. 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 respectively²⁵.

Dendrimers for controlled release drug delivery

Frechet 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. PEG-2000 was conjugated to generation G3 PAMAM dendrimers with varying degree of substitution. Methotrexate drug was encapsulated (loaded) to the prepared conjugates and investigated for drug release in a dialysis bag. The results found that PEG-dendrimers conjugated with encapsulated drug and sustained release of methotrexate as compare to unencapsulated drug. Controlled release of the Flurbiprofen could be achieved by formation of complex with amine terminated generation 4 (G4) P AMAM dendrimers. Prepared dendrimer complexes observed that loaded drug displayed initial rapid release (more than 40% till 3rd hour) followed by slow release. Pharmacodynamic study was performed using carrageenan induced paw edema model, revealed 75% inhibition at 4th hour that was maintained above 50% till 8th hour. The dendritic formulation showed 2-fold and 3-fold increased in mean residence time and terminal half-life, respectively, as compared to free drugs²⁶.

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. The use of dendrimers as gene transfection agents and drug-

delivery devices have been extensively reviewed part. The ability of cationic dendrimers to deliver DNA or RNA has been reviewed previously. Besides of that some research recently indicated that dendrimer based gene delivery system also have significant potential in clinical trials. Compared to conventional means such as viral vectors, biolistic particle bombardment, electroporation or polyethylene glycol attachment, and these dendrimer complexes having a many advantages such as less invasive, improves transgenic efficiency and could dramatically enhance the procedures for crop improvement using transgenic technology²⁷.

Dendrimers as imaging agents

The first in vivo diagnostic imaging applications using dendrimer-based MRI contrast agents were demonstrated in the early 1990s. 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 with gadolinium groups (Magnevist[®]). These generation dependent, dramatic enhancements of MRI contrast properties were some of the first examples of a dendritic effect. Gadolinium is an FDA-approved contrast agent for MRI which provides greater contrast between normal tissue and abnormal tissue in the brain and body. It is safer than the iodine type contrast used in CT scans and also non-radioactive and is rapidly cleared by kidneys. The largest MRI contrast agent G5 with 64 Gd (III) ions gives lowest concentration detection limit which would make G5 potentially the best dendritic MRI contrast agent²⁸.

Conclusion

Although dendrimeric 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. Therefore, the future work on this is expected to address the formidable challenges encountered in the field of drug targeting, and opens up new vistas in future development of a promisingly active and site-specific delivery system.

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