

A REVIEW ON DENDRIMERS: A NOVEL APPROACH**Hemant Rathod*, Saurabh Patel, Dilip Agrawal, M.P.Khinchi****Department of Pharmaceutics, Kota College of Pharmacy, Kota, Rajasthan, India.****Abstract:**

Dendrimers are novel class of approach in polymers. They are monodisperse macromolecules having extensively branched 3D structure which provides a high degree of surface functionality and versatility. They are used for biological and drug-delivery applications because of their unique properties like uniform size, high degree of branching, water solubility, multivalency, well-defined molecular weight and available internal cavities. The aim of this review to describe about dendrimer synthesis strategies, types of dendrimers with different functionalities, properties which having crucial importance and their potential applications.

Keywords: Dendrimer, Convergent, Divergent, PAMAM

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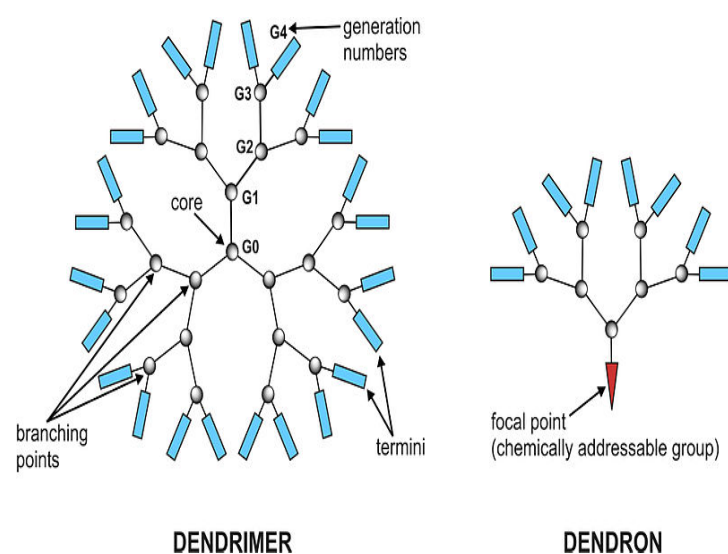
Introduction:

Nanoparticles have unusual properties that can be used to improve drug delivery. Where larger particles would have been cleared from the body, cells take up these nanoparticles because of their 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).¹

Dendrimers is the class of synthetic polymeric macromolecule which play important role in emerging nanotechnology. It is described as highly branched macromolecule, which provides a high degree of surface functionality and versatility. It is derived from the Greek words *Dendron* (tree) and *meros* (part)². It possess a 3-D features that resemble a tree. Dendrimer is an internationally accepted term and other synonymous terms are ‘arborols’ and ‘cascade molecules’. The first ever dendrimers were described by Vögtle in 1978³. Then in early 1980s pioneering work was done by Denkewalter

and co-workers as polylysine dendrimers at Allied Corporation⁴, Tomalia et al. at Dow Chemical on dendrimer synthesis^{5,6,7}. Dendrimers are large and complex molecules having very well-defined chemical structures. Dendrimers are built from a starting atom, such as nitrogen, to which carbon and other elements are added by a repeating series of chemical reactions that produce a spherical branching structure. As the process repeats, successive layers are added. It possesses three distinguished architectural components as shown in fig. 1⁸.

1. An initiator core.
2. Interior layers (generations) composed of repeating units, radically attached to the interior core.
3. Exterior (terminal functionality) attached to the outermost interior generations.



[Figure-1 Structure of Dendrimer]

Dendrimers are generally produced in an iterative sequence of reaction steps, in that each additional iteration leads to higher generation dendrimer. Each of the new layer 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, too.

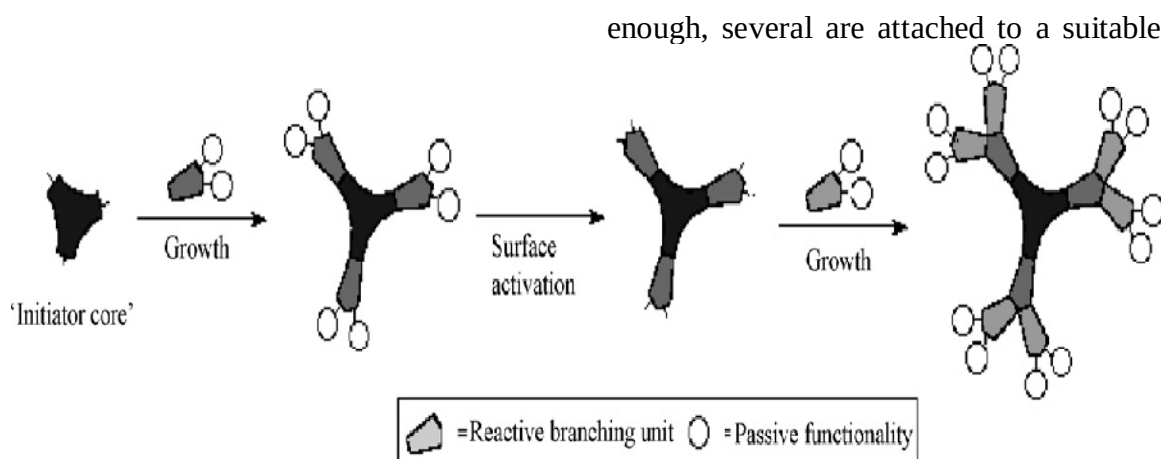
A dendrimer is typically symmetric around the core, and often adopts a spherical three-dimensional morphology. The word dendron is also encountered frequently. A dendron usually contains a single chemically addressable group called the focal point. The difference between dendrons and dendrimers is illustrated in figure one, but the terms are typically encountered interchangeably.⁹

Synthesis of Dendrimers

Dendrimers are generally prepared using either a divergent method or a convergent one¹⁰. There is a fundamental difference between these two construction concepts.

Divergent growth method: A schematic representation of divergent growth is shown in Fig. 2. In this method, 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. The subsequent liberated reactive sites lead to the first

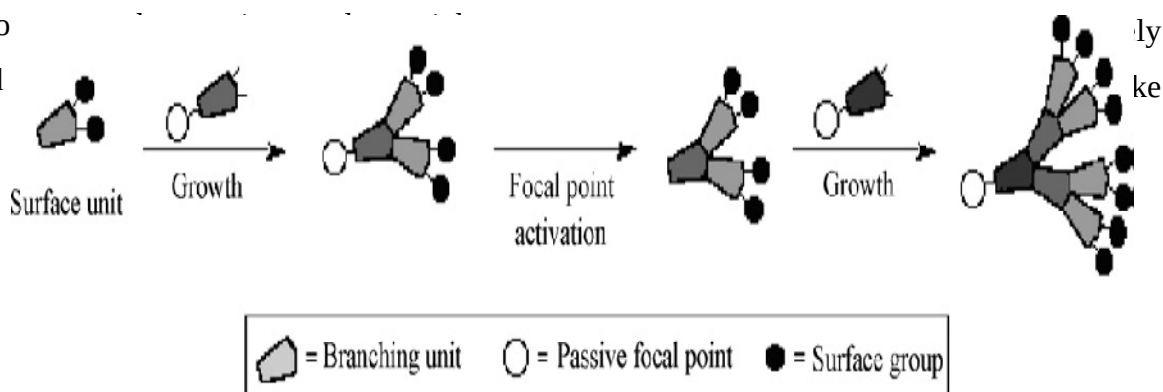
generation dendrimers. This process is repeated until the dendrimer of the described size is obtained¹¹. PAMAM dendrimers are synthesized by divergent growth approach. This method involves in situ construction in stepwise, iterative stages around a desired core such as ethylenediamine, ammonia or cystamine to produce well-defined structure. The two step reaction sequences exposed on a core consists of (a) an exhaustive alkylation of primary amines (Michael addition) with methyl acrylate, and (b) amidation of amplified ester groups with a large excess of ethylenediamine to produce primary amine terminal groups which creates 'generation zero' (G0) of the dendrimer series. Each amino group present in this branched molecule is then subsequently reacted with two additional molecules of methylmethacrylate monomer, followed by reaction with two more ethylenediamine molecules, to provide first generation starburst dendrimer^{8, 11}. Most divergent dendrimer synthesis requires excessive monomer loading and lengthy chromatographic separations, particularly at higher generations. This divergent approach is currently the preferred commercial strategy used by worldwide producers; such as Dendritic Nanotechnology Incorporation, DSM and Prestorp.



[Figure-2 Divergent growth method.]

Convergent growth method: This is an alternative approach to dendrimer synthesis. The difficulty of many reactions that have to be performed on one molecule is overcome by performing a small number of reactions on a large number of molecules. The growing dendrons are large and

reactive sites are functionalized in each step, giving a small number of possible side reactions per steps. The other



[Figure-3 Convergent growth method.]

begins at what will end up being the surface of the dendrimer, and work inwards by gradually linking surface units together with more monomers Fig. 3. When the growing dendrons are large

positions and numbers. The deactive products are easier to separate by purification at each synthesized generation

of dendrimer hence the products are more homogeneous although purification in the higher generation dendrons becomes more cumbersome, because of increasing similarity between reactants and formed products. 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 core¹³. The convergent methodology also suffers from low yields in the synthesis of large structures.

Properties of Dendrimers

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 viruses¹⁴. In the report by Shaunak et al., the polyvalency of a dendrimer glucosamine conjugates is also thought to be responsible for the ability of these molecule to efficiently inhibit scar-tissue formation. In preliminary animal studies, the bifunctional dendrimer increased the long-term success rate of glaucoma surgery from 30% to 80%¹⁵.

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)¹⁶. 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. That is the reason, Tomalia said that convergently produced dendrimers are probably the most precise synthetic macromolecules that exist today

⁸. However, mass spectroscopy has shown that dendrimers produced by divergent method are remarkably monodisperse for earlier generation ($G = 0-5$) ¹⁷. 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 (i.e. $G > 4$) by special techniques like azeotropic (n-butanol) and wiped film distillation ¹⁸.

Nanoscale size and shape: Based on their dimensional length scaling, narrow size distribution, and other biomimetic properties, dendrimers are often referred an “artificial proteins” ¹⁹. Within the PAMAM dendrimer family, the diameter of the generation 1–10 with ethylenediamine core increases from 1.1 to

12.4nm. 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 (G_0-G_3) with ethylenediamine core have ellipsoidal shapes, whereas the PAMAM dendrimer of higher generation (G_4-G_{10}) have roughly spherical shapes ²⁰. X-ray analysis on dendrimer aggregates have been revealed that the molecular shape of the lower to higher generations becomes increasingly globular (i.e. more spherical compare to linear shaped), in order to spread out the larger molecular structure with a minimal repulsion between the segments ²¹. These fundamental properties have in fact lead to their commercial use for gene therapy, immunodiagnostics and variety of other biological applications ²².

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 hyper branched 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 ²³. 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 “denaturated” (e.g. extended) conformations dependent on the polarity, ionic strength and pH of the solvent. 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 amore extended conformation with a highly expanded surface area ²⁴. Polarity of the solvent greatly influences the structure of the dendrimer. NMR studies performed on PPI dendrimer revealed that an apolar solvent such as benzene will favour polar intramolecular interactions (e.g. hydrogen bonding) resulting in back-folding of the dendrimer arms into the dendrimer interior, whereas the increased acidity of chloroform, will increase salvation of the dendrimeric structure via hydrogen bond donation to the interior tertiary amines resulting in a more extended conformation of the dendrimer ²⁵. At high ionic strength (high concentration of salts) charged PPI dendrimers favours a contracted conformation of dendrimers with a high degree of back-folding. In contrast at low salt concentration, the repulsive forces between the charged dendrimer segments result in an extended conformation ²⁴.

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.

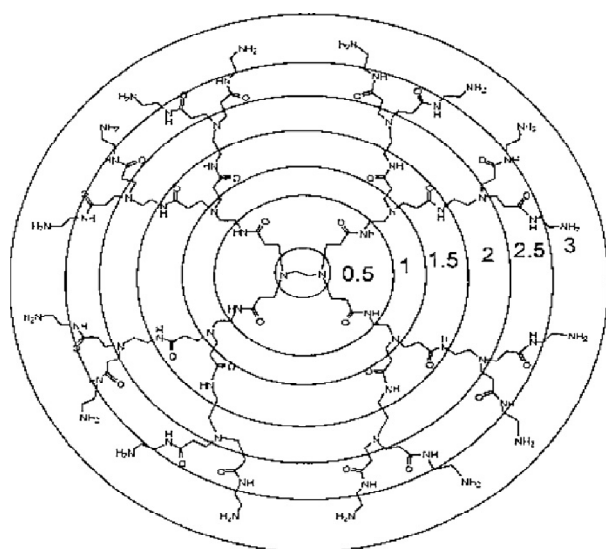
Till today, the cytotoxicity of dendrimers has been primarily studied in vitro; however, a few in vivo studies have been reported. Duncan and co-workers have investigated the relationship between structure and biocompatibility of PAMAM, poly (propyleneimine), poly (ethylene oxide) grafted carbosilane dendrimers with cationic (NH₂-terminated) and anionic (COONa-terminated) dendrimers in vitro. They have reported that, regardless of structure, cationic dendrimers were generally more haemolytic and cytotoxic effect at even relatively low concentration in comparison to anionic dendrimers. They have also reported increase in the haemolysis with increase in the generation²⁶. One more study reported that anionic PAMAM dendrimers have shown a significantly lower cytotoxicity than

cationic dendrimer using Caco-2 cells²⁷. Hydroxy- or methoxy-terminated polyester dendrimers have been reported to have low toxicity in both in vitro and in vivo studies. During in vitro study, at high concentration, these dendrimers have induced some inhibition of cell growth but no increase in cell death was observed. Upon injection into mice, no acute or long-term toxicity problems observed. The results make these new dendritic motifs promising candidates for drug-delivery devices²⁸. Immunogenicity is one of the crucial biological property 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 dendrimers²⁹.

Types of Dendrimers

PAMAM dendrimer: Perhaps the family of dendrimers most investigated for drug delivery is the polyamidoamine (PAMAM) dendrimer (Fig. 4). 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 temperatures³⁰. 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 up to generation 10 (G10) have been obtained. PAMAM dendrimers are the most extensively reported moiety for almost all existing applications of dendrimers.



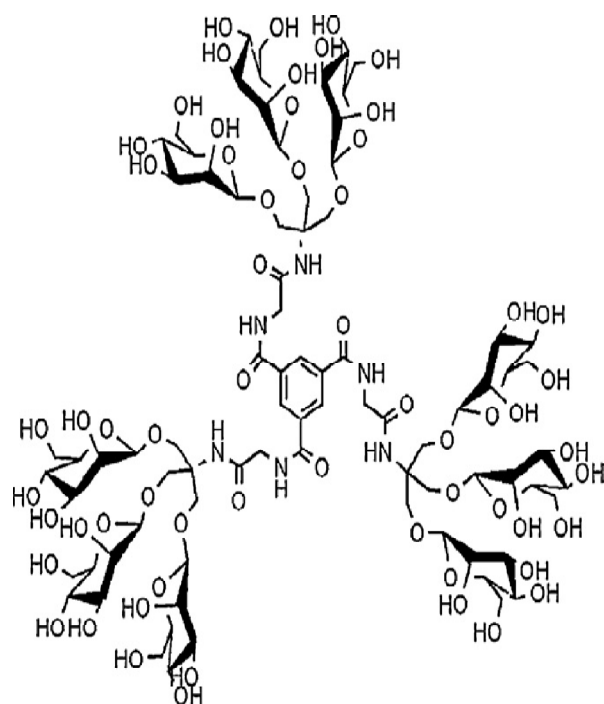
[Figure-4 PAMAM generation 3 dendrimer]

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 OS domains³¹.

Glycodendrimers: The term 'glycodendrimers' is used to describe dendrimers that incorporate carbohydrates into their structures (Fig. 5). 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 beneficial^{32, 33}.

Peptide dendrimers: Dendrimers having peptides on the surface of the traditional dendrimer frame work and dendrimers incorporating amino acids as branching or core units are both defined as 'peptide dendrimers'³². Also peptide dendrimers can be defined as macromolecules that



[Figure-5 Carbohydrate containing glycodendrimers]

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 Ca²⁺ 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 serodiagnosis^{34, 35}.

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 polymers¹¹.

Applications of Dendrimers

Dendrimers in gene delivery: The ability to deliver pieces of DNA to the required parts of a cell includes many challenges. Current research is being performed to find ways to use dendrimers to traffic genes into cells without damaging or deactivating the DNA. To maintain the activity of DNA during dehydration, the dendrimer/DNA complexes were encapsulated in a water soluble polymer, and then deposited on or sandwiched in functional polymer films with a fast degradation rate to mediate gene transfection. Based on this method, PAMAM dendrimer/DNA complexes were

used to encapsulate functional biodegradable polymer films for substrate mediated gene delivery. Research has shown that the fast degrading functional polymer has great potential for localized transfection^{36, 37, 38}.

Dendrimers as Sensors: Scientists have also studied dendrimers for use in sensor technologies. Studied systems include proton or pH sensors using poly(propylene imine),³⁹ cadmium-sulfide/polypropylenimine tetrahexacontaamine dendrimer composites to detect fluorescence signal quenching,⁴⁰ and poly(propylenamine) first and second generation dendrimers for metal cation photodetection⁴¹ amongst others. Research in this field is vast and ongoing due to the potential for multiple detection and binding sites in dendritic structures.

Dendrimers as Blood Substitution: Dendrimers are also being investigated for use as blood substitutes. Their steric bulk surrounding a heme-mimetic centre significantly slows degradation compared to free heme^{42, 43}, and prevents the cytotoxicity exhibited by free heme.

Dendrimers for the synthesis of Nanoparticles: Dendrimers also are used in the synthesis of monodisperse metallic nanoparticles. Poly (amidoamide), or PAMAM, dendrimers are utilized for their

tertiary amine groups at the branching points within the dendrimer. Metal ions are introduced to an aqueous dendrimer solution and the metal ions form a complex with the lone pair of electrons present at the tertiary amines. After complexation, the ions are reduced to their zero valent states to form a nanoparticle that is encapsulated within the dendrimer. These nanoparticles range in width from 1.5 to 10 nanometers and are aptly called Dendrimer-Encapsulated Nanoparticles.⁴⁴

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^{45, 46, 47}. 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) PAMAM 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 drugs⁴⁸.

Dendrimers for Targeted Drug Delivery: Dendrimers have ideal properties which are useful in targeted drug-delivery system. The targeted delivery of chemotherapeutics to tumor cells reduced side effects compared to systemic delivery. Macromolecular delivery of anti-cancer drugs using multifunctional dendritic architectures allows for the conjugation of both drugs and targeting moieties such as folic acid,

monoclonal antibodies, and peptides to the dendrimer periphery for increasingly specific delivery. The two general strategies of targeting include the passive targeting of bulk cancerous tissue and the active targeting of unique tumor cells.

Non-specific or passive targeting of tumors is achieved by increasing the hydrodynamic radius of the dendrimer through Pegylation, leading to the accumulation of dendrimer in tumor tissue via the enhanced permeability retention (EPR) effect. The EPR effect is a result of tumor induced angiogenesis leading to neovasculature that is irregular, leaky or defective with disorganized endothelial cells; tumor tissues also suffer from poor lymphatic drainage, all leading to the accumulation and retention of macromolecules in the tumor mass. Specific or active targeting relies on the conjugation of one or more targeting moieties to the dendrimer to facilitate cell-receptor-mediated interactions⁴⁹.

Dendrimers for Pulmonary Delivery: Pegylated dendrimeric micelles prolong the half-life of low molecular weight heparin (LMWH), Enoxaparin and increase the drug's pulmonary absorption, thereby efficacious in preventing deep vein thrombosis (DVT) in a rodent model. Shuhua Bai have prepared dendrimers of LMWH entrapped in PEG these produced

a significant increase in pulmonary absorption and the relative bioavailability of the formulation was 60.6% compared to subcutaneous LMWH. The half-life of the PEG–dendrimer-based formulation was 11.9 h, which is 2.4-fold greater than the half-life of LMWH in a saline control formulation. When the formulation was administered at 48-h intervals, the efficacy of LMWH encapsulated in pegylated dendrimers in reducing thrombus weight in a rodent model was very similar to that of subcutaneous LMWH administered at 24-h intervals⁵⁰.

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 biodegradable⁵¹. 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 bioavailability⁵².

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 colleagues⁵³. 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 generation dependent, dramatic enhancements of MRI contrast properties were some of the first examples of a ‘dendritic effect’⁵⁴. 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 kidneys⁴⁷. Different generation G1 [n = 4 Gd (III) ions per molecule], G3 (n = 16), G5 (n = 64) of Gd

(III) DTPA-terminated poly (propylene imine) dendrimers and Gd (III) DTPA complex [G0 (n = 10)] reference were synthesized and investigated for relaxivities and concentration detection limits. Investigator revealed dendrimers G1, G3 and G5 have been shown an increase in both T1 and T2 relaxivities with increasing molecular weight. Relaxivity values, measured in blood plasma are in good agreement with earlier measurements in citrate buffers, it shows these dendritic contrast agents have very less interactions with blood plasma proteins. The largest MRI contrast agent G5 with 64 Gd (III) ions gives lowest concentration detection limit would make G5 potentially the best dendritic MRI contrast agent⁵⁵.

Conclusion:

Dendrimers is a novel approach in the pharmaceutical and biomedical industry. Dendrimers provide lots of benefits in many applications. Dendrimers still have toxicity problems but it can also be resolved by modification of their structure. Use of dendrimers in medical field is rising and future work is necessary to find out its cost effective production.

Reference:

1. Tomalia, D.A., Naylor, A.M., Goddard, W.A., 1990. Starburst dendrimers: molecular level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter. *Angew. Chem., Int. Ed. Engl.* 29, 138–175.
2. Breitenbach J. *Spektrum der Wissenschaft/Digest: Moderne Chemie* 1998:96–7.
3. Bhuleier, E., Wehner, W., Vogtle, F., 1978. “Cascade”- and “nonskid-chain-like” synthesis of molecular cavity topologies. *Synthesis* 1978, 155–158.
4. Denkwalter, R.G., Kolc, J., Lukasavage, W.J., 1981. Macromolecular highly branched homogeneous compound based on lysine units. US Patent 4,289,872
5. Tomalia, D.A., Baker, H., Dewald, J., Hall, M., Kallos, G., Martin, S., Roeck, J., Ryder, J., Smith, P., 1985. A new class of polymers: starburst-dendritic macromolecules. *Polym. J.* 17, 117–132.
6. Tomalia, D.A., Dewald, J.R., 1985. The Dow Chemical Company. Dense star polymers having core, core branches, terminal groups. US Patent 4,507,466.

7. Newkome, G.R., Yao, Z., Baker, G.R., Gupta, V.K., 1985. Micelles. Part 1. Cascade molecules: a new approach to micelles. A [27]-arborol. J. Org. Chem. 50, 2003–2004.
8. Tomalia DA. Birth of a new macromolecular architecture – dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. Progress in Polymer Science 2005; 30:294–324.
9. Nanjwade, Basavaraj K.; Hiren M. Bechraa, Ganesh K. Derkara, F.V. Manvia, Veerendra K. Nanjwade (2009). "Dendrimers: Emerging polymers for drug-delivery systems". *European Journal of Pharmaceutical Sciences* (Elsevier) **38** (3): 185–196.
10. Hodge, P. (1993) Polymer science branches out. *Nature* **362**, 18–19.
11. Jain, N.K., Khopade, A.J., 2001. Dendrimers as potential delivery systems for bioactives. In: Jain, N.K. (Ed.), *Advances in controlled and novel drug delivery*. CBS Publishers & Distributors, New Delhi, pp. 361–380.
12. Matthews, O.A., Shipway, A.N., Stoddart, J.F., 1998. Dendrimers—branching out from curiosities into new technologies. *Prog. Polym. Sci.* 23, 1–56.
13. Fréchet, J.M.J., Tomalia, D.A., 2001. *Dendrimers and Other Dendritic Polymers*. Wiley, Chichester.
14. Halford, B., 2005. Dendrimers branch out. *Chem. Eng. News* 83, 30–36.
15. Shaunak, S., Thomas, S., Gianasi, E., Godwin, A., Jones, E., Teo, I., Mireskandari, K., Luthert, P., Duncan, R., Patterson, S., Khaw, P., Brocchini, S., 2004. Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. *Nat. Biotechnol.* 22, 977–984.
16. Jackson, J.L., Chanzy, H.D., Booy, F.P., Drake, B.J., Tomalia, D.A., Bauer, B.J., Amis, E.J., 1998. Visualization of dendrimer molecules by transmission electron (TEM): staining methods and Cryo-TEM of vitrified solutions. *Macromolecules* 31, 6259–6265.
17. Tomalia, D.A., 1993. Starburst/cascade dendrimers: fundamental building blocks for a new nanoscopic chemistry set. *Aldrichim. Acta* 26, 91–101.
18. Maciejewski, M., 1982. Concept of trapping topologically by shell

- molecules. *J. Macromol. Sci., Chem.* 17, 689.
19. Svenson, S., Tomalia, D.A., 2005. Dendrimers in biomedical applications-reflections on the field. *Adv. Drug Deliv. Rev.* 57, 2106–2129.
20. Cheng, Y., Zhenhua, X., Minglu, M., Tongwen, X., 2008. Dendrimers as drug carriers: applications in different routes of drug administration. *J. Pharm. Sci.* 97, 123–143.
21. Percec, V., Cho, W.D., Mosier, P.E., Ungar, G., Yeardley, D.J.P., 1998. Structural analysis of cylindrical and spherical supramolecular dendrimers quantifies the concept of monodendron shape control by generation number. *J. Am. Chem. Soc.* 120, 11061–11070.
22. Bieniarz, C., 1998. Dendrimers: applications to pharmaceutical and medicinal chemistry. In: *Encyclopedia of Pharmaceutical Technology*. Marcel Dekker, New York, pp. 55–89.
23. Farin, D., Avnir, D., 1991. Surface fractality of dendrimers. *Angew. Chem., Int. Ed. Engl.* 30, 1379–1380.
24. Boas, U., Christensen, J.B., Heegaard, P.M.H., 2006. *Dendrimers in Medicine and Biotechnology: New Molecular Tools*. Royal Society of Chemistry, Cambridge.
25. Chai, M., Niu, Y., Youngs, W.J., Rinaldi, P.L., 2001. Structure and conformation of DAB dendrimers in solution via multidimensional NMR techniques. *J. Am. Chem. Soc.* 123, 4670–4678.
26. Malik, N., Wiwattanapatapee, R., Klopsch, R., Lorenz, K., Frey, H., Weener, J.W., Meijer, E.W., Paulus, W., Duncan, R., 2000. Dendrimers: relationship between structure and biocompatibility in vitro and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. *J. Control. Release* 65, 133–148.
27. Jevprasesphant, R., Penny, J., Jalal, R., Attwood, D., McKeown, N.B., D'Emanuele, A., 2003a. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. *Int. J. Pharm.* 252, 263–266.
28. Padilla De Jesu's, O.L., Ihre, H.R., Gagne, L., Fre'chet, J.M.J., Szoka Jr., F.C., 2002. Polyester dendritic

- systems for drug delivery applications: in vitro and in vivo evaluation. *Bioconjug. Chem.* 13, 453–461.
29. Kobayashi, H., Kawamoto, S., Saga, T., Sato, N., Hiraga, A., Ishimori, T., Konishi, J., Togashi, K., Brechbiel, M.W., 2001. Positive effects of polyethylene glycol conjugation to generation-4 polyamidoamine dendrimers as macromolecular MR contrast agents. *Magn. Reson. Med.* 46, 781–788.
 30. Lee, C.C., MacKay, J.A., Fréchet, J.M.J., Szoka, F.C., 2005. Designing dendrimers for biological applications. *Nat. Biotechnol.* 23, 15-17-1526.
 31. Dvornic, P.R., de Leuze-Jallouli, A.M., Owen, M.J., Perz, S.V., 2000. Radially layered poly (amidoamine-organosilicon) dendrimers. *Macromolecules* 33, 53–66.
 32. Colinger, M., 2002. Biological applications of dendrimers. *Curr. Opin. Chem. Biol.* 6, 742–748.
 33. Turnbull, W.B., Stoddart, J.F., 2002. Design and synthesis of glycodendrimers. *Rev. Mol. Biotechnol.* 90, 231–255.
 34. Bruckdorfer, T., Marder, O., Albericio, F., 2004. From production of peptides in milligram amounts for research to multi-tons quantities for drugs of the future. *Curr. Pharm. Biotechnol.* 5, 29–43.
 35. Crespo, L., Sanclimens, G., Pons, M., Giralt, E., Royo, M., Albericio, F., 2005. Peptide and amide bond-containing dendrimers. *Chem. Rev.* 105, 1663–1681.
 36. Fu, Hui-Li; Si-Xue Cheng, Xian-Zheng Zhang, Ren-Xi Zhuo (2008). "Dendrimer/DNA complexes encapsulated functional biodegradable polymer for substrate-mediated gene delivery". *The Journal of Gene Medicine* (Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, Wuhan, People's Republic of China) **10** (12): 1334–1342.
 37. Fu, HL; Cheng SX, Zhang XZ (2007). "Dendrimer/DNA complexes encapsulated in a water soluble polymer and supported on fast degrading star poly (DL-lactide) for localized gene delivery". *Journal of Control Release* (Key Laboratory of Biomedical Polymers of Ministry

- of Education, Department of Chemistry, Wuhan University) **124** (3): 181–188.
38. Tathagata Dutta, Minakshi Garg, and N.K.Jain. "Poly(propyleneimine) dendrimer and dendrosome based genetic immunization against Hepatitis B". *Vaccine*. 2008. 26(27-28): 3389-3394.
 39. Fernandes, Edson G. R.; Vieira, Nirton C. S.; de Queiroz, Alvaro A. A.; Guimaraes, Francisco E. G.; Zucolotto, Valtencir. (2010). "Immobilization of Poly(propyleneimine) Dendrimer/Nickel Phtalocyanine as Nanostructured Multilayer Films To Be Used as Gate Membranes for SEGFET pH Sensors". *Journal of Physical Chemistry C* (American Chemical Society) **114** (14): 6478–6483.
 40. Campos, Bruno B; Algarra, Manuel; Esteves da Silva, Joaquim C. G (2010). "Fluorescent Properties of a Hybrid Cadmium Sulfide-Dendrimer Nanocomposite and its Quenching with Nitromethane". *Journal of Fluorescence* (Springer) **20** (1): 143–151.
 41. Grabchev, Ivo; Staneva, Desislava; Chovelon, Jean-Marc (2010). "Photophysical investigations on the sensor potential of novel, poly(propyleneimine) dendrimers modified with 1,8-naphthalimide units". *Dyes and Pigments* (Elsevier Ltd.) **85** (3): 189–193.
 42. Twyman, L. J.; Ge, Y. (2006). "Porphyrin cored hyper branched polymers as heme protein models". *Chemical Communications* (15): 1658.
 43. Twyman, L. J.; Ellis, A.; Gittins, P. J. (2012). "Pyridine encapsulated hyper branched polymers as mimetic models of heme containing proteins that also provide interesting and unusual porphyrin-ligand geometries". *Chemical Communications* **48** (1): 154–156.
 44. Crooks, Richard; Scott, Wilson (September 2005). "Synthesis, Characterization, and Applications of Dendrimer-Encapsulated Nanoparticles". *American Chemical Society* (109): 692-704.
 45. Patri, A.K., Majoros, I.J., Baker Jr., J.R., 2002. Dendritic polymer macromolecular carriers for drug delivery. *Curr. Opin. Chem. Biol.* 6, 466–471.

46. Liu, M., Kono, K., Fréchet, J.M.J., 1999. Water-soluble dendrimer-poly(ethylene glycol) starlike conjugates as potential drug carriers. *J. Polym. Sci. Part A: Polym. Chem.* 37, 3492–3503.
47. Liu, M., Kono, K., Fréchet, J.M.J., 2000. Water-soluble dendritic unimolecular micelles: their potential as drug delivery agents. *J. Control. Release* 65, 121–131.
48. Asthana, A., Chauhan, A.S., Diwan, P.V., Jain, N.K., 2005. Poly (amidoamine) (pamam) dendritic nanostructures for controlled site specific delivery of acidic anti-inflammatory active ingredient. *AAPS PharmSciTech.* 6, Article 67.
49. Iyer AK, Khaled G, Fang J and Maeda H, Exploiting the enhanced permeability and retention effect for tumor targeting, *Drug Discovery Today* 2006;11: 812–818.
50. Shuhua B and Fakhrul A. Synthesis and Evaluation of Pegylated Dendrimeric Nano carrier for Pulmonary Delivery of Low Molecular Weight Heparin. *Pharmaceutical Research* 2004; 26(3): 539-548.
51. Tolia, G.T., Choi, H.H., 2008. The role of dendrimers in drug delivery. *Pharmaceut. Tech.* 32, 88–98.
52. Vandamme, T.F., Brobeck, L., 2005. Poly (amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. *J. Control. Release* 102, 23–38.
53. Wiener, E.C., Brechbiel, M.W., Brothers, H., Magin, R.L., Gansow, O.A., Tomalia, D.A., Lauterbur, P.C., 1994. Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents. *Magn. Reson. Med.* 31, 1–8.
54. Tomalia, D.A., Reyna, L.A., Svenson, S., 2007. Dendrimers as multi-purpose nanodevices for oncology drug delivery and diagnostic imaging. *Biochem. Soc. Trans.* 35, 61–67.
55. Langereis, S., Lussanet, Q.G., van Genderen, M.H.P., Meijer, E.W., Beets-Tan, R.G.H., Griffioen, A.W., van Engelshoven, J.M.A., Backes, W.H., 2006. Evaluation of Gd (III) DTPA-terminated poly (propylene imine) dendrimers as contrast agents for MR imaging. *NMR Biomed.* 19, 133–141.