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# DENDRIMERS: THE POTENTIAL HAULER IN GENE THERAPY

**Sheetal K. Medhekar[1*], Namdeo G. Shinde[2], Riyaz Ali Osmani[2], Dhanashri U. Gadhave[2], Suyog P. Sulake[2], Shraddha M. Kumbhar[2]**

[1]Department of Pharmacology, Satara College of Pharmacy, Satara (M. S.), India, 415 004.

[2]Department of Pharmaceutics, Satara College of Pharmacy, Satara (M. S.), India, 415 004.

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***Correspondence for Author:**
**Sheetal K. Medhekar**,
Department of Pharmacology,
Satara College of Pharmacy,
Satara (M. S.), India, 415 004

## ABSTRACT

Gene therapy is the technique in which defective or non-functional genes are replaced with targeted gene. Gene therapy has gained significant attention over the past two decades as a potential method for treating genetic disorders such as severe combined immunodeficiency, cystic fibrosis and Parkinson's disease. Viral and on-viral vectors commonly used for gene delivery. Each of these vectors is designed to deliver normal copies of a gene into cells that contain only a mutated copy or defective gene. Viral vectors have been found to be dangerously toxic and this was tragically demonstrated when a 18 year old boy enrolled in gene therapy study had a massive immune reaction to the virus used resulting in death in only a few days due to multiple organ failure. This article chiefly focuses on the treatment of genetic disease by using activated polymer (dendrimers) and by use of siRNA technology. These approaches were made to deliver the drug to the targeted site and thus preventing toxicity and side effects to other cells and also reduce the chances of death in case of viral mediated gene transfer.

**Keywords:** Dendrimers, Gene therapy, Activated dendrimers, siRNA technology.

## INTRODUCTION

### Dendrimers

Dendrimers initially named as starburst polymer are synthetic, highly branched, mono-disperse macromolecules of nanometer dimensions with a three-dimensional structure. The name comes from the Greek word δένδρον (dendron), which translates to "tree". Synonymous terms for dendrimer include *arborols* and *cascade molecules*[1].

These are large and complex molecules with very well-defined chemical structures. From a polymer chemistry point of view, dendrimers are nearly perfect monodisperse (basically meaning of a consistent size and form) macromolecules with a regular and highly branched three dimensional architecture. They consist of three main architectural components: core, branches and end groups[2].

**General Structure:** Structure of dendrimers consist of three different parts (**Fig. 1**)-

1) Initiator core,

2) Interior layer (generation) - contain repeating units radially attached to initiator core,

3) Exterior layer- attached to outermost interior generation layer.

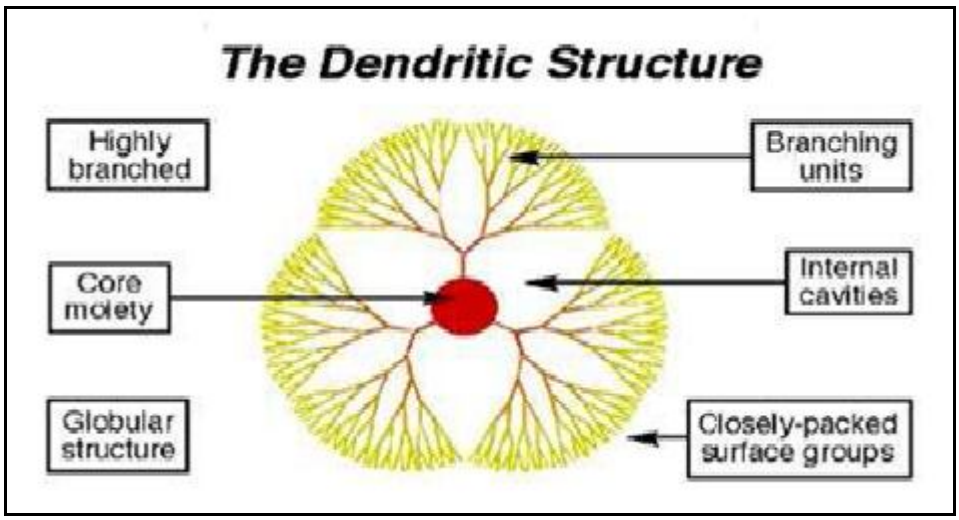


**Fig. 1: Structure of dendrimers.**

Dendrimers are produced in an iterative sequence of reaction steps, in which each additional iteration leads to a higher generation dendrimer (**Table No. 1**). The creation of dendrimers, using specifically designed chemical reactions is one of the best examples of controlled hierarchical synthesis, an approach that allows the 'bottom up' creation of complex system. Each new layer creates a new 'generation', with double number of active sites (called end groups) and approximately double the molecular weight of previous generation. One of the most appealing aspects of technologies based on dendrimers is that it is relatively easy to control their size, composition and chemical reactivity very precisely[2,3].

**Table No. 1: Generation and corresponding structure of dendrimers.**

| Generation of dendrimers | Structure |
|---|---|
| **First three** | Small, not specific three dimensional structure |
| **Fourth (G4)** | Beginning to become spherical, to a preferred three dimensional structure |
| **Fifth (G5)** | Highly structured spheres |

**Types of Dendrimers**

**1) PAMAM Dendrimers**

Poly (amidoamine) dendrimers (PAMAM) are synthesized by the divergent method starting from ammonia or ethylenediamine initiator core reagents. Products up to generation 10 (a molecular weight of over 9,30,000 g/mol) have been obtained (by comparison, the molecular weight of human hemoglobin is approximately 65,000 g/mol). PAMAM dendrimers are commercially available, usually as methanol solutions. *Starburst dendrimers* is applied as a trademark name for a sub-class of PAMAM dendrimers based on a tris-aminoethylene-imine core. The name refers to the star like pattern observed when looking at the structure of the high-generation dendrimers of this type in two-dimensions[4-6].

**2) 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 domains.

**3) Tecto Dendrimers**

These are composed of a core dendrimer, surrounded by dendrimers of several steps (each type design) to perform a function necessary for a smart therapeutic nanodevice. Different compounds perform varied functions ranging from diseased cell recognition, diagnosis of disease state drug delivery, reporting location to reporting outcomes of therapy.

**4) Multilingual Dendrimers**

In these dendrimers, the surface contains multiple copies of a particular functional group.

**5) Chiral Dendrimers**

The chirality in these dendrimers is based upon the construction of constitutionally different but chemically similar branches to chiral core.

**6) Hybrid Dendrimers Linear Polymers**

These are hybrids (block or graft polymers) of dendritic and linear polymers.

**7) Amphiphilic Dendrimers**

They are built with two segregated sites of chain end, one half is electron donating and the other half is electron withdrawing.

**8) Multiple Antigen Peptide Dendrimers**

It is a dendron like molecular construct based upon a polylysine skeleton. Lysine with its alkyl amino side-chain serves as a good monomer for the introduction of numerous of branching points. This type of dendrimer was introduced by *JP Tam* in 1988, has predominantly found its use in biological applications like e.g. vaccine and diagnostic research[5,7,8].

**9) PPI Dendrimers**

PPI-dendrimers stand for "Poly (Propylene Imine)" describing the propylamine spacer moieties is the oldest known dendrimer type developed initially by *Vogtle*. These dendrimers are generally poly-alkyl amines having primary amines as end groups. The dendrimer interior consists of numerous of tertiary tris-propylene amines. PPI dendrimers are commercially available up to G5, and has found widespread applications in material science as well as in biology. As an alternative name to PPI, POPAM is sometimes used to describe this class of dendrimers. POPAM stands for Poly (Propylene Amine), which closely resembles the PPI abbreviation. In addition, these dendrimers are also sometimes denoted as "DAB-dendrimers" where DAB refers to the core structure, which is usually based on diamino butane.

**10) Micellar Dendrimers**

These are unimolecular micelles of water soluble hyper branched polyphenylenes.

**11) Frechet-Type Dendrimers**

It is a more recent type of dendrimer developed by *Hawker* and *Frechet* based on poly-benzyl ether hyper branched skeleton. These dendrimers usually have carboxylic acid groups as surface groups, serving as a good anchoring point for further surface functionalisation, and as polar surface groups to increase the solubility of this hydrophobic dendrimer in polar solvents or aqueous media[9-11].

**Specific Types of Dendrimers for Drug Delivery**

Dendrimers most widely investigated for drug delivery is the polyamidoamine (PAMAM) dendrimer. PAMAM dendrimers are biocompatible, non-immunogenic, water-soluble and

possess terminal modifiable amine functional groups for binding to various targets or guest molecules synthesis. Drugs can be delivered either by encapsulation of drugs and/or by dendrimer-drug conjugates by making use of various dendrimers [12].

**Gene Therapy**

Gene therapy is a process by which defective or deficient gene is replaced by normal functioning gene to treat a number of diseases, such as severe combined immuno-deficiencies, hemophilia, Parkinson's disease, cancer and even HIV. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state[13]. Several approaches to gene therapy are being tested, including:

- ✓ Replacing a mutated gene that causes disease with a healthy copy of the gene,
- ✓ Inactivating, or "knocking out", a mutated gene that is functioning improperly,
- ✓ Introducing a new gene into the body to help fight a disease etc.

Process of gene therapy requires carriers/vectors either viral or non-viral. Use of dendrimers as a non-viral vector in gene therapy is widely used approach to treat various inheritable disease such as adenosine deaminase deficiency, cystic fibrosis, Gaucher's disease and Duchenne muscular dystrophy and some acquired disease like AIDS, cancer etc.

Before discussing how dendrimer in nanotechnology are used to aid gene it is important to have a thorough understanding of the conventional mechanism of gene therapy and the limitations of this procedure in the prevention of genetics diseases[14,15].

In conventional therapy targeting of drugs can be achieved by changing the physicochemical properties of a drug like particle size, molecular weight, surface charge but also making use of specific ligand antibodies or carbohydrates. In targeted drug delivery system in case of gene therapy a functional gene to replace defective gene is transfected to targeted cell/site i.e. to be treated (**Fig. 2** and **3** depicts viral mediated gene transfer).

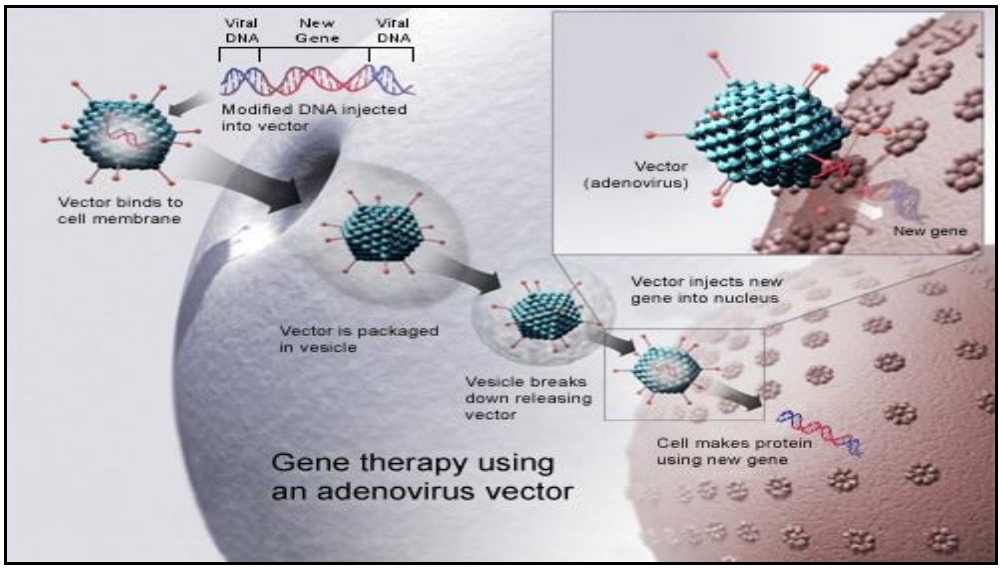


**Fig. 2: Viral vector mediated gene therapy.**

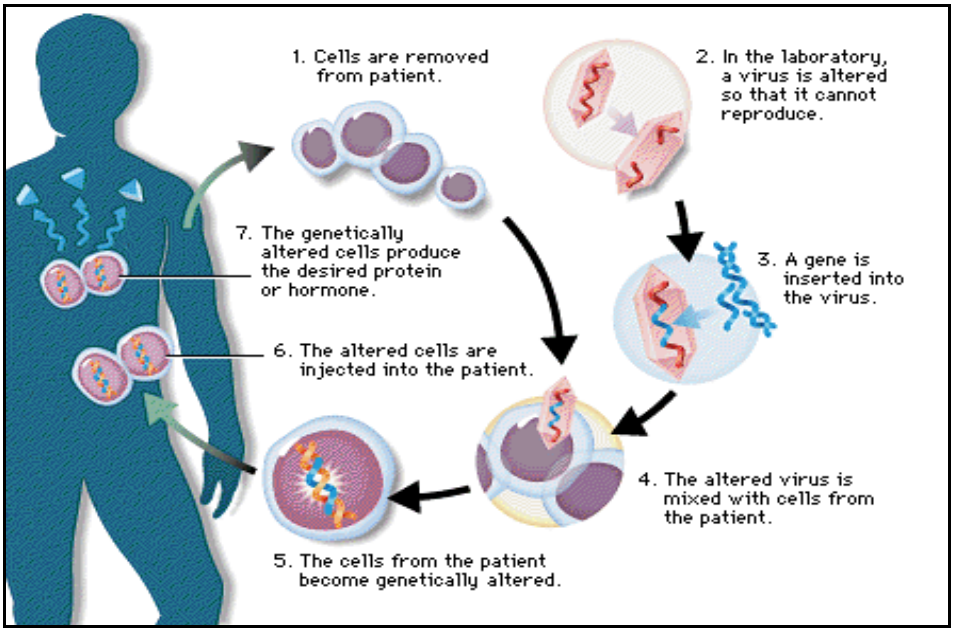


**Fig. 3: Gene therapy using a viral vector.**

**Disadvantage of Viral Mediated Gene Therapy**

1. Toxicity of viral vector includes high inflammatory activity and immunogenicity, integration into host genome and mutation to and/or contamination of wild type virus.

2. In addition, preparation and up scaling of vector production are difficult to achieve for some viral vector including retrovirus and adeno associated virus (AAV)[16].

3. Viral vectors have been found to be dangerously toxic and this was tragically demonstrated when a 18 year old boy enrolled in gene therapy study had a massive immune reaction to the virus used resulting in death in only a few days due to multiple organ failure.

Therefore non-viral vector become attractive alternative with decreased immunogenicity. Non-viral methods include physical methods such as electroporation, microinjection, gene gun, impalefection, hydrostatic pressure, continuous infusion, and sonication and chemical, such as lipofection. It can also include the use of polymeric gene carriers (polyplexes)[17].

**Dendrimers as Gene Carrier**

Gene transfection is a direct approach where large macromolecules, such as plasmid DNA for on-viral gene therapy is coupled to a nanoparticle of inert solid; which is then directly targeted to the cell nucleus[6]. The ideal vector as non-viral gene transfer agents for transfection should have high efficiency, non-immunogenic, non-toxic, either bio-degradable or excretable and also have long blood circulation time. The use of dendrimers for transfection was first reported by the group of *Szoka et al.*[7] and *Baker et al.*[8]. PAMAM dendrimers were the first found to be useful for transfection. The company named "Quiagen" developed a commercial transfection system based on PAMAM dendrimers followed by the work of *Szoka et al.* and *Baker et al.*[7,8]. The use of amino terminated PAMAM or PPI dendrimers as non-viral gene transfer agents; enhance the transfection of DNA by endocytosis and ultimately, into the cell nucleus. Additionally these offer many advantages over other vectors (**Fig. 4**). For certain gene delivery operations, the dendrimers of high structural flexibility and partially degraded high generation dendrimers are better suited.

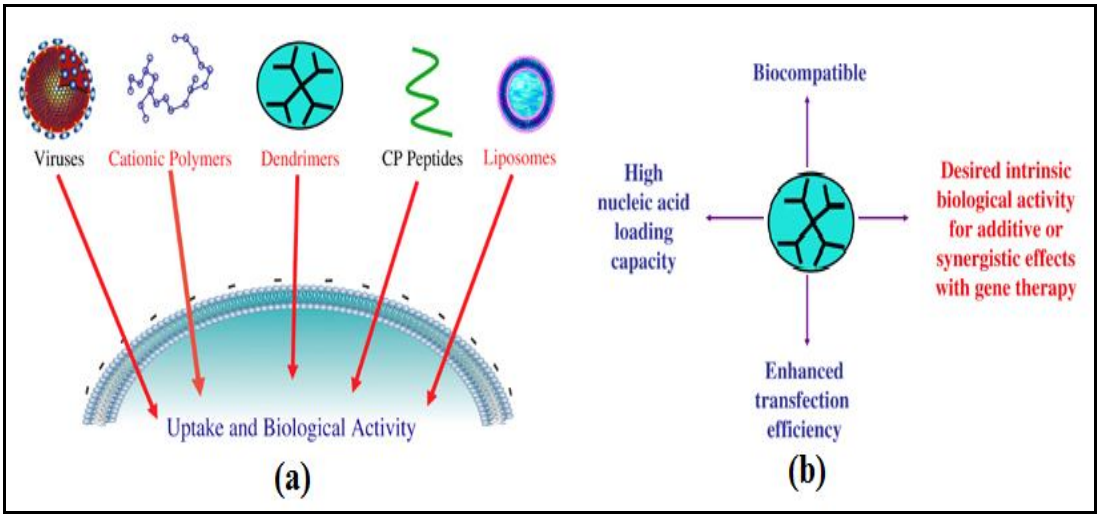


**Fig. 4: (a) Gene therapy using different vectors, (b) Significance of dendrimers mediated gene therapy.**

The suitability of any gene delivery system always has to be matched with the clinical situation, the specific disease and chosen therapeutic strategy. However, an optimum

molecular weight greater than 116 kDa was found best for PAMAM dendrimers which gave an optimum activity. In one of the study, dendritic amidoamine side chains of different generations were covalently attached to the chitosan; which was chosen to combine the biological activities of chitosan in gene delivery, antibacterial activity and wound healing activity with the delivery benefits found for dendrimers[8,12].

A number of different technologies to mediate gene transfer have certain drawbacks such as cytotoxicity, low efficiency and/or restricted applicability as listed in **Table No. 2**. Activated PAMAM dendrimers provide a new technology for gene transfer that offers significant advantages over classical methods. Reagents based on this technology provide high gene transfer efficiencies, minimal cytotoxicity and can be used with a broad range of cell types.

**Table No. 2: Different technologies to mediate gene transfer.**

| Method | Example | Limitation |
|---|---|---|
| Naked DNA | 1. Insertion of DNA coated catheter.<br>2. High pressure DNA coated gold particle. | Low level of expression. |
| Cationic liposomes | 1. Intranasal administration to target lungs of cystic fibrosis. | Difficulty in targeting. |
| Polymeric delivery systems | 1. Cationic polymers.<br>2. Anionic polymers. | Cannot condense DNA but protect naked DNA from degradation by endonuclease. |

**Gene Transfer into Eukaryotic Cells**

DNA is fundamentally a polymer of high molecular weight, typically in excess of 106 Da. It possesses a strong anionic charge conferred by its phosphate backbone. This combination of high molecular weight and surface charge restrict its passage across biological membranes, which consist predominantly of negatively charged lipid molecules. Thus, a key component of any delivery system is the modification of these properties in order to make the crossing of biological membranes more favorable. This can be achieved by the neutralization of the negative charge, which results in a reduction in the size of the DNA, as well as increasing its lipophilicity.

Gene transfer into eukaryotic cells involves two key steps (as depicted in **Fig. 5**):

1. Uptake of DNA by the cell, and
2. Transport of DNA into the nucleus.

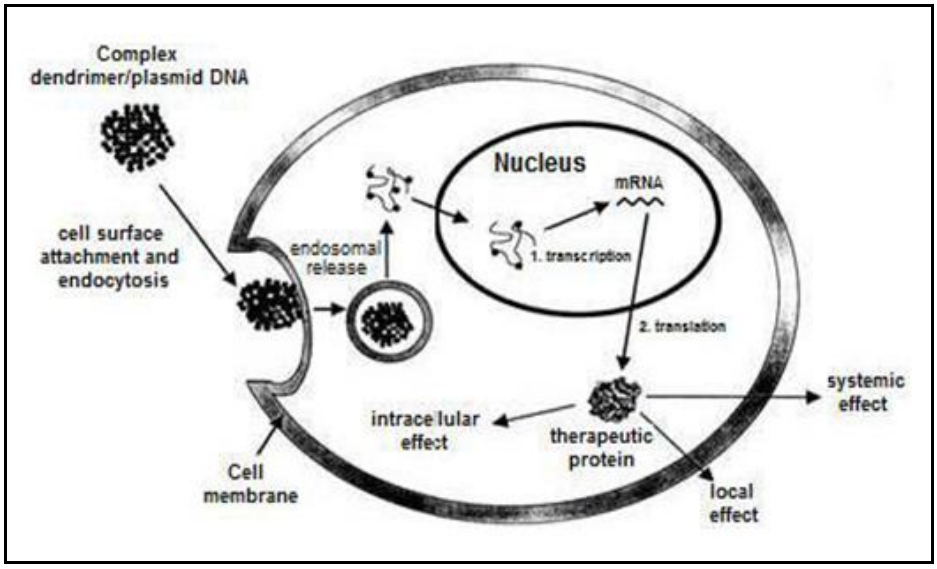


**Fig. 5: Entry of dendrimer-DNA complex into eukaryotic cell.**

A transfection technology should therefore provide an efficient way for DNA to enter a cell, and then protect the DNA from the cell's natural defense mechanisms until it has reached the nucleus. Depending on the application, the transfection technology should allow highly efficient gene transfer either into a broad range of cell types, or into a specific target cell type. There are also applications where specific transfection of only certain cell types is desirable. For example in gene therapy, specific transfection of the target tissue or cells avoids unwanted side effects in other tissues. The individual characteristics of different cell types play an important role in the establishment of transfection technologies for such applications. Thus, an ideal transfection technology would also provide flexibility in its cell specificity[18].

**Modern Transfection Technologies**

Liposome technology often offers higher transfection efficiencies and better reproducibility than DEAE-dextran and calcium phosphate methods. However, liposome reagents are often cytotoxic, and transfection results can vary between different cell types. Activated PAMAM-dendrimers condense DNA into compact transfection complexes that can adhere to the cell surface and be taken into the cell via endocytosis (**Fig. 6**), and offer significant advantages over other transfection technologies. These reagents are less cytotoxic than many other transfection technologies and provide higher transfection efficiencies than other classical technology (liposome-based) reagent, and can be used for the broad range of cells[19].

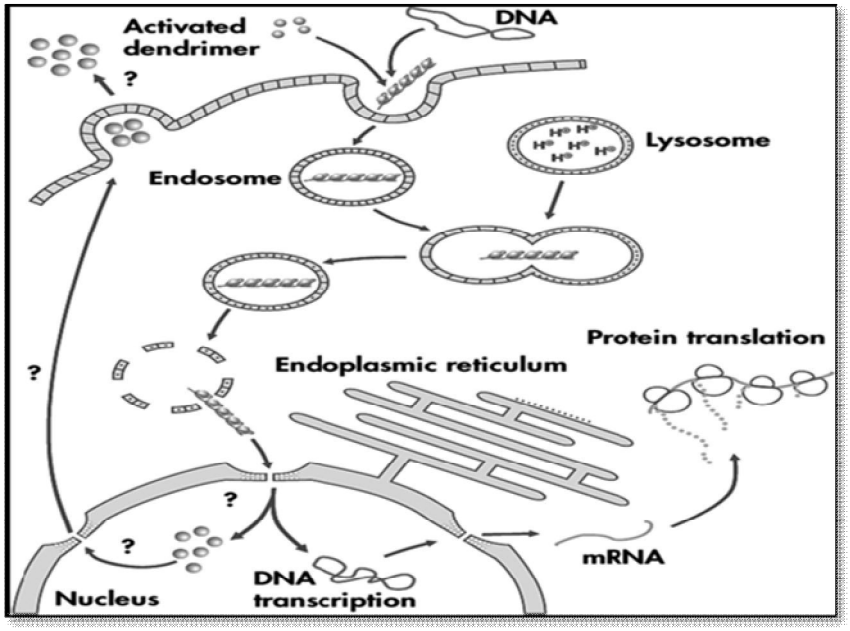


**Fig. 6: Model of activated dendrimer-mediated DNA uptake.**

In the first step of the transfection process, the DNA activated dendrimer complex binds to the surface of the cell. The complex is then taken into the cell by endocytosis, and incorporated into the endosome of the cell. From the endosome the DNA is released into the cytosol. A small percentage of the released DNA reaches the nucleus, where it is transcribed into RNA. In the last step the RNA is transported back into the cytosol and then translated into protein.

**Synthesis of Dendrimers**

Preferably two different methods used for stepwise synthesis of dendritic polymers are:

**1) 'Divergent' Dendrimer Growth Method**

This type of synthesis involves addition of branching monomer units repeatedly to produce a dendrimer of desired generation number. Starting from a reactive core, a generation is grown, and then the new periphery of the molecule is activated for reaction with more monomers. These two steps are repeated. This method is preferred for the production of large quantities of dendrimers since, in each generation-adding step, the molar mass of the dendrimer is doubled**.**

**2) 'Convergent' Dendrimer Growth Method**

In order to overcome disadvantages of divergent synthesis, the convergent approach was developed. Convergent growth begins at end up point, being the surface of the dendrimer, and works inwards by gradually linking surface units together with more and more units.

When the growing wedges are large enough, several are attached to a suitable core to give a complete dendrimer. The advantage of convergent growth over divergent growth is that only two simultaneous reactions are required for any generation adding step (**Fig. 7**)[20].

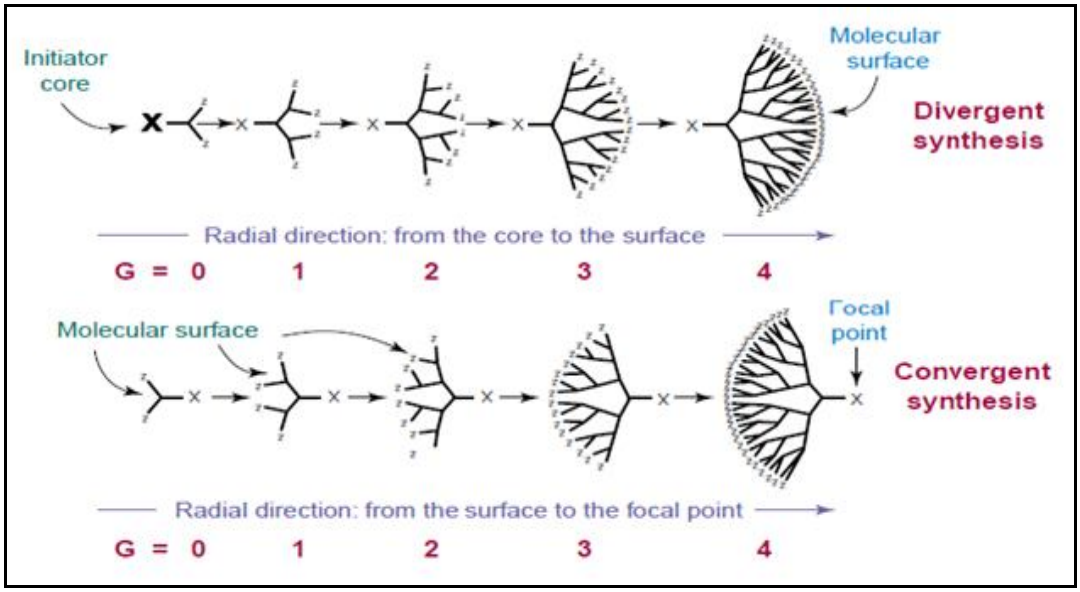


**Fig. 7: Schematic presentation of divergent and convergent synthesis methods.**

## PAMAM-dendrimers in Gene Therapy

### Synthesis of PAMAM-dendrimers

The dendrimers relevant for gene transfer have a multifunctional amine as the core moiety, and are synthesized by repeated Michael Addition of methylacrylate and reaction of the product with ethylenediamine by *Haensler* and *Szoka*, in 1993. The resulting PAMAM-dendrimers have alternating amido and amine bonds and are built up by layers of 'shells'. The different shells are called as generations. After generation four, steric factors cause PAMAM dendrimers to be spherical. The terminal amine groups give PAMAM-dendrimers a net positive charge at physiological pH 7-8. At this pH, both protonized and unprotonized amine groups are present. Generation six or seven PAMAM-dendrimers with a diameter of 6-10 nm and a molecular mass of 30-50 kDa are generally used for gene transfer[7,10].

### Activation of PAMAM-dendrimers

Newly synthesized PAMAM-dendrimers have a defined size and shape. Their activation can be achieved by solubilization in an appropriate solvolytic solvent and heating for a defined period of time leads to hydrolytic cleavage of some of the amido bonds in the inner part of the molecule and removal of some of the branches of the dendrimer (**Fig. 8**). Carboxyl groups form at the amido bond cleavage sites, and the molecular mass of the dendrimer is reduced by

20-25%. This process is called 'activation', which results in dendrimers with a higher degree of flexibility. Activation is a random process that gives a mixed population of dendrimer molecules differing slightly in molecular mass and structure. Overall size and shape of the dendrimer molecule, however does not change following activation. Both non-activated and activated dendrimers interact electrostatically with DNA to form DNA-dendrimer complexes that mediate gene transfer. In case of cultured eukaryotic cells, the transfection efficiency of activated dendrimers is 2-3 times higher than that of non-activated dendrimers[21]. This is due to the higher flexibility of activated dendrimers compared to the more rigid structure of newly synthesized non-activated dendrimers. This increased flexibility also plays a key role in the release of DNA from the endosome.

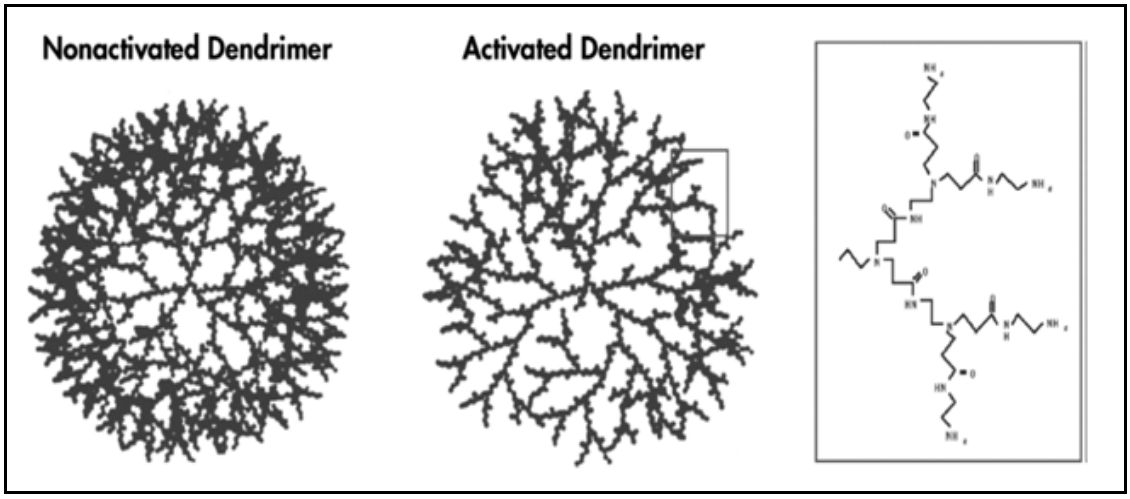


**Fig. 8: Schematic diagram of a non-activated (left) activated (middle) and magnified PAMAM-dendrimer branches.**

**Activated PAMAM Dendrimers Mediated Gene Transfer**

Activated PAMAM-dendrimers are mixed with the interested DNA for transfection. Positively charged amino groups on the surface of the dendrimer molecule interact with the negatively charged phosphate groups of the DNA molecule to form a DNA-dendrimer complex with a toroid-like structure having diameter of approximately 50-100 nm. There is a net positive charge on DNA-dendrimer complex and it can bind to negatively charged surface molecules on the membrane of eukaryotic cells. Complexes bound to the cell surface are taken into the cell by non-specific endocytosis. Once inside the cell, the complexes are transported to the endosomes. DNA is protected from degradation by endosomal nucleases by being highly condensed within the DNA-dendrimer complex (**Fig. 6**). In addition, amino groups on the dendrimers that are unprotonated at neutral pH can become protonated in the

acidic environment of the endosome. This leads to buffering of the endosome, which inhibits pH-dependent endosomal nucleases[11,22].

After discharge from the endosome, DNA must penetrate the nuclear membrane for transcription and subsequent expression to occur. While efficient transfection has been shown to depend on mitosis; when the nuclear envelope breaks down, there is also evidence that the nuclear pores act as a size-exclusion barrier. Small DNA fragments enter the nucleus by passive diffusion while larger fragments are transported through the nuclear pore complex in an energy-dependent manner[23]. Translocation into the nucleus does occur within 30 min post transfection.

The biotechnology company QIAGEN has developed two commercially available transfection reagents based on activated PAMAM-dendrimers, Super-Fect Transfection Reagent and Poly-Fect Transfection Reagent. These two reagents differ in their core moiety, generation number and activation procedure, and are successfully used in many laboratories for gene transfer experiments in different cell types. They attributed to increased flexibility in the structure. Addition of other agents to the DNA-dendrimer complex alters transfection. For example, chloroquine or dextran added to dendrimer-DNA complexes significantly increase transgene expression in a number of cell lines. DEAE-9 (diethyl amino ethyl) dextran is believed to alter the nature of the dendrimer by dispersing complex aggregates. However, it is cytotoxic and might prevent stable gene integration. Complexing DNA with dendrimers changes the molecular structure of DNA and results in condensation and aggregation of DNA particles, but also increases DNA survival upon delivery *in-vitro* and *in-vivo*[24]. Complex formation analysis and characterization has been carried out on soluble-insoluble or low-high density particles by various methods such as UV light absorption, laser light scattering and measurements that use radiolabelled DNA and/or dendrimers[25]. The actual binding affinity constants of DNA and dendrimers are not easily determined, partly because of the subsequent aggregation and precipitation of the complexes[26].

**Dendrimers in siRNA Technology**

The small or short interfering RNA (siRNA) technology has also gained a wide use during last few years in treatment of various genetic diseases. In this technique small interfering RNA (siRNA) with 19-21 base pairs is used as therapeutic agent for effectively silencing a disease-related gene on a post-transcriptional level. Translational process of disease causing protein is inhibited by siRNA technology. Hence, specific targeted cancer or HIV infected

cells can be treated with siRNA. Carriers for siRNA are divided broadly into two categories: viral and non-viral. Non-viral carriers typically involve complex of siRNA with positively charged vectors such as cationic polymers[27,29].

Although the commercially available dendrimers Polyfect and Superfect are designed for the delivery of plasmids, they have also been used for the delivery of short interfering RNA(siRNA), siRNA delivered to HeLa cells using Polyfect achieved silencing of the target genes by 90% as determined by the absence of the target proteins P120RasGAP and p130Cas. Superfect-mediated delivery of siRNA achieved knockdown results by more than 50% of the target Erbin protein that acts in the localization and signaling of ERBB-2 receptor in epithelia in rat pheochromocytoma derived (PC12) cells. More recently, the polyamine siPORT (Applied Biosystems, Carlsbad, California) has been specifically designed for siRNA delivery. A knockdown of 90% of both focal adhesion kinase (FAK) mRNA and protein was achieved when siPORT was used to deliver siRNA to human pancreatic ductal adenocarcinoma cells[30,31].

## CONCLUSIONS

Dendrimers interact with all forms of nucleic acids such as DNA, RNA and antisense oligonucleotides by electrostatic interaction, to form stable complexes which compact the nucleic acids and protect against degradation by nucleases. Hence, dendrimers has found extensive therapeutic applications in delivery of nucleic acids nowadays. Apart, dendrimer based delivery systems have shown considerable, promising tools for the newfangled development of gene therapies. Thus, many approaches are been urbanized for delivering interested gene to the targeted site without degradation so as to treat various life threatening genetic disorders and also to treat acquired diseases like AIDS, cancer etc.

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