Available online on 15.03.2016 at http://jddtonline.info
Journal of Drug Delivery and Therapeutics
An International Peer Reviewed Journal
Open access to Pharmaceutical and Medical research

REVIEW ARTICLE

POTENTIAL APPLICATION OF DENDRIMERS IN DRUG DELIVERY: A CONCISE REVIEW AND UPDATE

[1]Parajapati Sunil Kumar, [2]Maurya Sheo Datta, [2]Das Manas Kumar, [2]Tilak Vijay Kumar, [3]Verma Krishna Kr, *[4]Dhakar Ram C,

[1]Dept. of Pharmacy, Bundelkhand University, Jhansi, India
[2]Dept. of Pharmacy, IEC Group of Institution, Greater Noida, India
[3]Ram-Eish Institute of Pharmacy, Greater noida, India
[4]Jhalawar Medical College & Hospital, Jhalawar, India

*Corresponding Author's Email: dhakar_rc@yahoo.co.in

Received 22 Jan 2016; Review Completed 29 Feb 2016; Accepted 06 March 2016, Available online 15 march 2016

ABSTRACT:

This review gives concise information about the application of dendrimers in the field of drug delivery. Due to their unique architecture these have improved physical and chemical properties. Due to their terminal groups these show high solubility, miscibility and reactivity. Dendrimers have well defined size, shape, molecular weight and monodispersity. These properties make the dendrimers a suitable carrier in drug delivery application. Dendrimers are unimolecular miceller in nature and due to this enhances the solubility of poorly soluble drugs. Their compatibility with DNA, heparin and polyanions make them more versatile. Dendrimers, also referred as modern day polymers, they offer much more good properties than the conventional polymers. Due to their multivalent and mono disperse character dendrimers have stimulated wide interest in the field of chemistry biology, especially in applications like drug delivery, gene therapy and chemotherapy. Self assembly produces a faster means of generating nanoscopic functional and structural systems. But their actual utility in drug delivery can be assessed only after deep understanding of factors affecting their properties and their behavior *in vivo*.

Key words: Dendrimers, Drug targeting, nanoscale carriers.

INTRODUCTION

Dendrimers are the new artificial macromolecules which have the structure like a tree. They are class of well defined hyperbranched synthetic polymer systems, which can be conjugated to various chemical species, such as detection agents, imaging agents, targeting components, biomolecules, pharmaceutical/therapeutic agents, radio ligands, affinity ligands, for various bioanalytical applications. The term "Dendrimer" arise from two Greek word; "Dendron" meaning tree and "Meros" meaning part. A typical dendrimer structure consists of three basic components: a multi-functional central core moiety where other molecules can be trapped [1, 2], branched units that emanates from the central core and external capping-groups. The highly regular branching units are organized in layers called "generations", and represent the repeating monomer unit of these synthetic macromolecules[3]. Therefore, dendrimers can be synthesized from simple branched monomer units, in a precise and controlled fashion from trunk to branch and to leaf "surface groups".

The three-dimensional structure of dendrimers gives them a variety of unique properties, such as nanoscaled globular shape, well-defined functional groups at the periphery, hydrophobic or hydrophilic cavities in the interior and extremely low polydispersity [4], and thus a wide range of potential applications.

They are nanoparticles and so has advantages over microparticles or others due to its small size, easy uptake by cells (through endocytosis) and thus brings drug "bound" to dendrimers into the cell [4, 5]. They are branched macromolecules have a central core unit having a high degree of molecular uniformity, narrow molecular weight, distribution, specific size and shape characteristics, and a highly- functionalized, terminal surface. The manufacturing process is a series of repetitive steps generating shells, starting with a central initiator core. Each subsequent shell represents a new "generation" of polymer with a larger molecular diameter, twice the number of reactive surface sites, and approximately double the molecular weight of the preceding generation.

The precise control over the distribution of drugs is highly valuable to abolish the typical drawbacks of

traditional medicine. In recent years, improved pharmacokinetics, biodistribution and controlled release of the drug to the specific targeted site has been achieved with polymer based drug delivery[6] Unlike traditional polymers, dendrimers have received considerable attention in biological applications due to their high water solubility,[7] biocompatibility,[8] polyvalency[9] and precise molecular weight.[3] These features make them an ideal carrier for drug delivery and targeting applications. For investigating dendrimers as drug delivery vehicles, their biopermeability across the biological membranes should be considered. In this review, we report on the noteworthy scientific advances and most recent literature, dealing various application of dendrimer in the field of drug delivery.

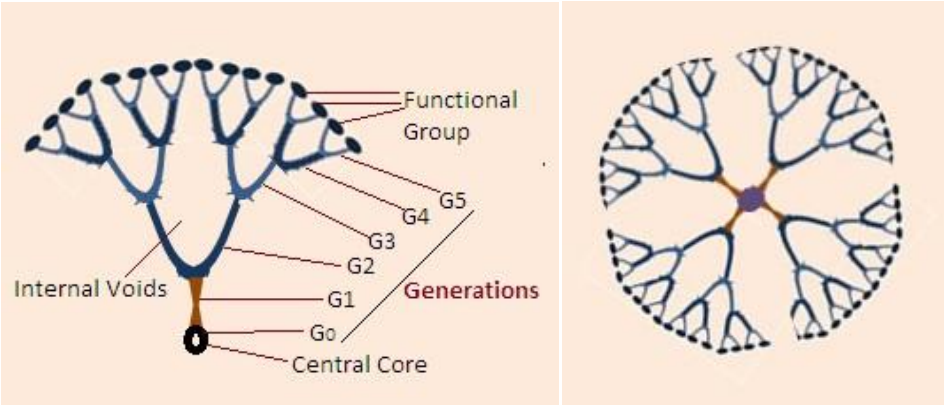

Figure 1: Schematic representation of the Dendrimer Structure. Adopted from reference [10]

WHAT MAKES DENDRIMERS SPECIAL IN DRUG DELIVERY

Dendrimers are dentritic polymers that have very well-defined nanostructures and high level control over its size, branching density and surface functionality. They are useful nanoscale carriers for drug and gene delivery. Both hydrophilic and phrophobic drug molecules can be formulated with dendrimers. They have been applied in intravenous, oral, pulmonary, nasal, ocular, and transdermal drug delivery systems.

Dendrimers have shown enormous potential as nanocarrier/delivery systems because they can cross cell barriers by both paracellular and transcellular pathways. The ability to statistically modify and optimize the number and/or ratio of dendrimer surface groups that influence biodistribution, receptor-mediated targeting, therapy dosage or controlled release of drugs from the dendrimer interior.

The three-dimensional structure of dendrimers gives them a variety of unique properties, such as nanoscaled globular shape, well-defined functional groups at the periphery, hydrophobic or hydrophilic cavities in the interior and extremely low polydispersity [11], and thus a wide range of potential applications. For example, most dendrimers have globular structures with molecular diameters less than 10 nm, which can be modulated by varying dendrimer generations.

This property gives dendrimers similar sizes and shapes as specific proteins and other biomolecules and thereby makes them perfect as biomimics [12]. Also, the highly regular branching pattern of dendrimers imbues these dendritic architectures with well-defined numbers of periphery functional groups, providing opportunities for the presence of drug molecules, targeting moieties, and solubilizing groups on the surface in a multivalent fashion [13, 14]. Moreover, hydrophobic and hydrophilic cavities in the interior of dendrimers make them useful candidates as unimolecular micelles for the encapsulation of guest molecules, especially drugs [15-16]. Finally, the low polydispersity of dendrimers assures the reproducibility of biodistribution of polymeric prodrugs using them as scaffolds [17-18].

Nanoparticles having a size in the range 1–10 nm have the capacity to diffuse into tumor cells. This helps to overcome limitations relating to chemotherapy using free drug such as poor in vivo/in vitro correlation and overcome other possible resistances offered by tumors.

Dendrimers are one of the most useful non-viral gene delivery systems. Their ability to transfect cells without inducing toxicity and be tuned for stimuli-induced gene delivery confers a great advantage over other gene delivery vectors for use in vivo.

The well-defined hyperbranched structure of dendrimers has motivated chemists to explore the possibility for mimicking protein functions with dendritic macromolecules, such as O2-carrying haemoproteins and coenzyme B12. Thus, dendrimers can be tuned for: (i) be stimuli-responsive nanocarriers, (ii) include moleculartags, (iii) possess high payload efficiency, (iv) decrease dosage requirements as well as re-dosage frequency and (v) target delivery and minimize drug migration, thus suppressing secondary effects during drug treatment.

The interesting nanoscale architecture of dendrimers confers several structural benefits over linear polymers, larger nanoparticles and liposomes. Such advantages include rapid cellular entry, reduced macrophage uptake, targetability and more facile passage across biological

barriers by transcytosis[19]. In comparison to linear polymers, dendrimers are multivalent owing to the presence of high multiplicities of reactive surface end groups, making them ideal drug carriers with higher drug payload capacities[20]. Encapsulation of drugs in PEGylated dendrimers can lead to enhanced permeation and retention (EPR) of the drug.

They can be synthesized and designed for specific applications. Due to their feasible topology, functionality and dimensions, they are ideal drug delivery systems; and also, their size is very close to various important biological polymers and assemblies such as DNA and proteins which are physiologically ideal.[21-22]

The covalent attachment of drugs to the surface groups of dendrimers through hydrolysable or biodegradable linkages enhances the pharmacological properties of the drug and offers the opportunity for a greater control over drug release.

Dendrimers are capable of improving the solubility, biodistribution, and efficacy of a number of therapeutics as well as being used as imaging and diagnostic molecules in animal models bearing brain tumors. These therapeutics can be conjugated to the surface via tumor-labile (pH sensitive) linkers or encapsulated noncovalently into the structure via electrostatics.

Table 1: Summary of the advantages of Dendrimers

- Improved bioavailability
- Overcoming of cellular barriers
- Cite specific Drug delivery
- Controlled drug release
- Better patient compliance
- Increased solubility, stability, and permeability of drugs
- Reduced macrophage uptake
- Facile passage across biological barriers by transcytosis
- Improved delivery efficiency
- The capability to deliver a variety of drugs
- Viral diagnosis
- Ability to maintain drug levels in a therapeutically desirable range
- Increased half-life
- Increased drug retention and providing extended therapeutic effects (e.g. Ocular drug delivery)
- Reduced side effects by targeted delivery
- Low toxicity and low immunogenicity
- High uniformity and purity
- Rapid cellular entry

MECHANISMS OF DRUG DELIVERY VIA DENDRIMERS:

Dendrimers are particularly attractive as they offer a high drug-loading capacity. Due to the well defined 3D structure and many surface functional groups, drug molecules can be loaded both in the interior of the dendrimers as well as attached to the surface groups. Dendrimers can function as drug carriers either by encapsulating drugs within the dendritic structure or by interacting with drugs at their terminal functional groups via electrostatic or covalent bonds forming prodrug. Encapsulation of drugs and dendrimer –drug conjugates[23-24] are two main methods of dendrimer drug delivery.

1. Non-covalent Encapsulation of Drugs / Host – Guest Relation

Incorporation of small organic molecules may be a result of non‑bonding interactions with specific groups within dendrimer, i.e. just physical entrapment

Encapsulation of drugs uses the satiric bulk of the exterior of the dendrimer or Interactions between the dendrimer and drug to trap the drug inside the dendrimer. Such a system can be used to encapsulate drugs and provide controlled delivery. Initial studies of dendrimer as potential delivery systems focused on their use as unimolecular micelles and ‘dendritic boxes’ for the noncovalent encapsulation of drug molecules. For example, in early studies, DNA was complexed with PAMAM dendrimers for gene delivery applications, and hydrophobic drugs and dye molecules were incorporated into various dendrimer cores. An advantage of using dendritic unimolecular micelles rather than conventional polymeric micelles is that the micellar structure is maintained at all concentrations because the hydrophobic segments are covalently connected[25].

Dendrimers can be used as dendritic boxes and unimolecular micelles (dendrimer‑drug networks) for the incorporation of hydrophobic/hydrophilic molecules by host guest interactions inside their empty cavities (nanoscale containers) present around core.[26-27] Jansen et al. were the first to entrap the rose bengal dye molecules in PPI dendrimers by using tert‑butyloxycarbonyl (t‑Boc) groups and led to the production of stable dendritic box that possess the bulky amino groups on the dendrimer surface.[28]

The dendritic unimolecular micelles contain the hydrophobic cores surrounded by hydrophilic shells and they offer an advantage over conventional polymeric micelles such that the micellar structure is maintained at all the concentrations because the hydrophobic segments are covalently connected.

2. Covalent Dendrimer–Drug Conjugates

Alternatively, the exploitation of well‑defined multivalent aspect of dendrimers allows the attachment of drug molecules to its periphery that result in

complex formation. The resultant complexes are formed either due to the electrostatic interactions between the drug and dendrimer or conjugation of the drug to dendrimer molecule. Through electrostatic interactions, various ionizable drugs form complexes with the large number of ionizable terminal surface groups of dendrimers.[29]

In dendrimer–drug conjugates, the drug is attached through a covalent bond either directly or via a linker/spacer to the surface groups of a dendrimer. Dendrimers have been conjugated to various biologically active molecules such as drugs, antibodies, sugar moieties and lipids .The drug loading can be tuned by varying the generation number of the dendrimer, and release of the drug can be controlled by incorporating degradable linkages between the drug and dendrimer. Moreover, the drugs can be covalently conjugated to dendrimers through some spacers that may include PEG, p‑amino benzoic acid, p‑amino hippuric acid and lauryl chains etc., or biodegradable linkages such as amide or ester bonds.

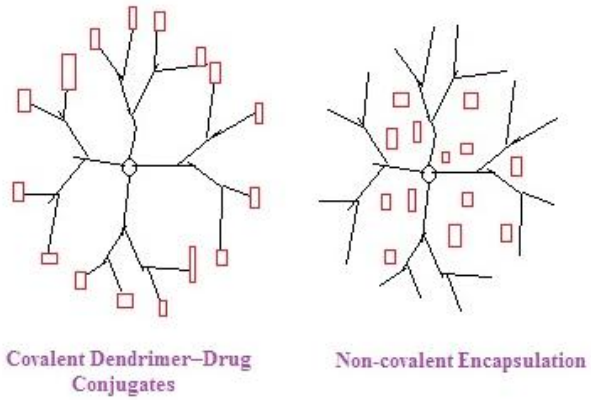

Figure 2: Different types of drug‑dendrimer interactions. Adopted from reference [10]

APPLICATION OF DENDRIMERS IN DRUG DELIVERY

Over the past 30 years greater attention has been focused on development of controlled and sustained drug delivery systems. Amongst the extensive research has been carried in designing of polymeric drug delivery systems[30].

The development of dendrimer based efficient drug delivery systems has attracted a great deal of attention over the last few years. Unlike traditional polymers, dendrimers can be obtained in precise molecular weights even at high generations, which as previously highlighted can provide a reproducible pharmacokinetic behavior. This feature makes them ideal candidates for drug delivery applications.

However, efficient drug delivery systems should meet other criteria, such as: (i) structural control over the size and shape of drug or imaging-agent cargo-space; (ii) biocompatibility, non-toxic polymer/pendant functionality; (iii) precise, nanoscale-container and/or scaffolding properties with high drug-loading capacity; (iv) well-defined scaffolding and/or surface modifiable functionality for cell specific targeting moieties; (v) lack of immunogenicity; (vi) appropriate cellular adhesion and internalization, (vii) adequate bio-elimination or biodegradation; (viii) controlled or stimuli-responsive drug release features; (ix) molecular level isolation and protection of the drug against inactivation during transit to target cells; (x) minimal non-specific cellular and blood–protein binding properties; (xi) ease of consistent, reproducible, clinical grade synthesis.

Figure 3: Various application of dendrimers in drug delivery

1. Dendrimers in CNS Drug Delivery

The brain is a challenging organ for drug delivery. First, the incidence of degenerative diseases in the brain will increase with the aging population. Secondly, the blood brain barrier (BBB) is well-known as the best gatekeeper in the body toward exogenous substances. All the current therapeutic strategies are not efficient in treating disorders related to the CNS[31]. Delivery of therapeutics to the brain is challenging because many organic molecules have inadequate aqueous solubility and limited bioavailability.

As the vast majority of potential CNS compounds have limited brain uptake, they may benefit from the use of advanced delivery systems in order to cross the BBB. The drug is encapsulated in, or associated to the particle, thereby masking its physiochemical characteristics[32].

Table 2: Rational design of dendrimers for *CNS Drug Delivery*

Dendrimers can offer many advantages over conventional drug delivery. They:	• **For rapid and effective clinical translation, the Dendrimer carrier should:**
• improve the delivery of therapeutics to the CNS by bypassing or crossing the BBB • protect the drug from premature degradation; • enhance absorption of the drugs into a selected tissue (for example, solid tumour); • control the pharmacokinetic and drug tissue distribution profile; • have ability to maintain drug levels in a therapeutically desirable range increased solubility, stability, and permeability of drugs, • have low toxicity and low immunogenicity	• be made from a material that is biocompatible, well characterized, and easily functionalized; • not only transport across the BBB but also target specific cells • pass the membrane and deliver the drug held in its interior • Have potential to overcome inadequate aqueous solubility and limited bioavailability of drugs • Require deeper toxicological studies • Engineered properly in order to enable their long term use without the accumulation of adverse effects

BBB is essential for maintaining a healthy brain; it impedes efforts to deliver therapeutic agents into the brain. The poor permeability of various drugs as well as delivery systems across the BBB is primarily due to tight junctions, lack of capillary fenestrations and presence of efflux transporters. The BBB can reportedly block more than 98% of CNS drugs[33]. Due to the ineffectiveness of conventional drug therapies, finding ways to deliver therapeutic drugs to the CNS safely and effectively is essential. The development of novel strategies that could overcome the obstacles of brain drug delivery is essential. The application of nanoscience to CNS disorders is an active area of research.

Nanomedicine has shown great potential for the treatment of many CNS diseases. Nanomedicine is the biomedical and pharmaceutical application of nanotechnology for making nanocarriers of therapeutics and imaging agents, nanoelectronic biosensors, and nanodevices with nanostructures. A number of nanocarrier delivery systems, including dendrimers, liposomes, polymeric micelles, linear polymers, quantum dots, and iron oxide Nanoparticles have been developed and have demonstrated promising properties in CNS drug deliver[34].

Among these, much attention has been paid to dendrimers because of their advantages, which include (1) the ability to maintain drug levels in a therapeutically desirable range, (2) increased half-life, (3) increased solubility, stability, and permeability of drugs, (4) the capability to deliver a variety of drugs, (5) reduced macrophage uptake, (6) targeting ability, (7) facile passage across biological barriers by transcytosis, (8) rapid cellular entry, (9) improved delivery efficiency, (10) reduced side effects by targeted delivery, (11) low toxicity and low immunogenicity, and (12) high uniformity and purity.[19, 35-36]

The presence of a large number of surface groups provides opportunity to conjugate ligands not only for transport across the BBB but also for target- ing specific cells, such as tumors. Dendrimers can be prepared with specific surface modifications that enable the dendrimers to gain entry through a membrane while holding a molecule that cannot pass on its own. Once the dendrimer passes the membrane, it can deliver the drug held in its interior.

The mechanism of uptake and toxicity to the BBB has not been extensively studied. A detailed characterization of dendrimer toxicity is important for the design and use of dendrimers in brain drug delivery. Toxicity of both the functional group and generation of the dendrimer must be taken into consideration. PAMAM dendrimers have been shown to be haemolytic and cytotoxic, with toxicity tending to be higher for cationic PAMAM dendrimers and to increase with generation[8,37].

Heather et al evaluated the potential toxicity of biotinylated G4 PAMAM dendrimer conjugates. Biotin is an important molecule used in several metabolic pathways and belongs to a family of molecules that have been shown to cross the BBB. The biophysical interactions of biotinylated G4 PAMAM conjugates and G4 PAMAMs with lipid model membranes were evaluated using Langmuir Blodgett monolayer techniques and atomic force microscopy (AFM). Results were correlated with cellular toxicity measurements using endothelial cell culture models of the BBB. This work reports the first analysis of PAMAM dendrimers using this combined approach. The results provide important insights into strategies for developing nanoparticle systems for brain drug delivery[38].

Delivery of therapeutics to the brain is challenging because many organic molecules have inadequate aqueous solubility and limited bioavailability. Katare et al investigated the efficiency of a dendrimer-based formulation of a poorly aqueous soluble drug, haloperidol, in targeting the brain via intranasal and intraperitoneal administration. Aqueous solubility of haloperidol was increased by more than 100-fold in the developed formulation. Formulation was assessed via different routes of administration for behavioral (cataleptic and locomotor) responses, and for haloperidol distribution in plasma and brain tissues. Dendrimer-based formulation showed significantly higher distribution of haloperidol in the brain and

plasma compared to a control formulation of haloperidol administered via intraperitoneal injection. Additionally, 6.7 times lower doses of the dendrimer−haloperidol formulation administered via the intranasal route produced behavioral responses that were comparable to those induced by haloperidol formulations administered via intraperitoneal injection. This study demonstrates the potential of dendrimer in improving the delivery of water insoluble drugs to brain [39].

Polyesters based dendrimers have been proposed for CNS regenerative medicine. Dhanikula et al. [40-41] suggested the use of polyether-copolyester (PEPE) dendrimers conjugated with d-glucosamine, and loaded with methotrexate (MTX) in order to allow a better delivery across the BBB. The results revealed that the efficacy of MTX-loaded dendrimers was established against U87 MG and U 343 MGa cells (two glioma cell lines). In vitro studies revealed that glucosylated dendrimers were internalized by endocytosis in significantly higher amounts than non-glucosylated dendrimers by both the cell lines. Moreover, the amount of MTX-transported across an in vitro model of the BBB was three to five times more after loading in the dendrimers, which indicates that glucosylation further increased the cumulative permeation of dendrimers across BBB, and hence increased the amount of MTX available across it. This work evaluated a different set of dendrimers, as well as different strategy, for potential use delivery across the BBB. However, it should be considered with caution, as only in vitro models were used, and the in vivo proof of concept is yet to be demonstrated.

Prieto et al. [42] investigated the cytotoxicity of sulfadiazine complexed with fourth-generation PAMAM dendrimers. Cell culture studies using fibroblasts (Vero cells) and macrophages (J-774 cells) revealed that the dendrimeric sulfadiazine complexes did not affect membrane integrity at low concentrations (0.031M). Moreover, cytotoxicity tests using human intestinal adenocarcinoma cell line (Caco-2 cells) showed that dendrimeric sulfadiazine did not reduce viability of Caco-2 cells over the tested concentrations as compared to that for PAMAM (G4). Remarkably, the in vivo study has shown that brain and muscle of Wistar rats are the main targets of intravenous administration of dendrimeric sulfadiazine, which can be advantageous for drug delivery applications directed to central nervous system.

However, challenges still exist regarding the deeper toxicological studies, specific targeting, and noninvasive alternative drug administration methods. The ultimate goal of dendrimer-mediated CNS drug-delivery systems is to engineer the dendrimers to be safe and to enable their longterm use without the accumulation of adverse effects.

2. Dendrimers in Oral Drug Delivery

The oral route of drug delivery in general is considered the favourite means of drug administration[43, 44]. Despite of tremendous innovations in drug delivery, the oral route remains the preferred route for administration of therapeutic agents because of accurate dosage, low cost therapy, self medication, non invasive method and ease of administration leading to high level of patient compliance.[45]

Dendrimers are suitable candidate in oral drug delivery because dendrimers loosened the tight junctions of epithelial layer and thus an improvement in the absorption of small molecular weight drugs was achieved [46]. The transepithelial transport and toxicity of polyamidoamine dendrimers as carriers for oral drug delivery has been reviewed by Sadekar and Ghandehari [47]

Kolhe et al. [48] demonstrated that ibuprofen predominantly forms a complex with PAMAM (G3 and G4) dendrimers because of the ionic interaction between the –NH2 end groups and the carboxyl group of ibuprofen. In this work, they demonstrated that the in vitro release of ibuprofen from drug–dendrimer complex is appreciably slower compared to pure ibuprofen. Moreover, the FITC-labeled dendrimer-complexed drug enters human lung epithelial carcinoma A549 cells much faster than pure drug, suggesting that dendrimers may be able to carry the complexed drug inside cells efficiently.

In another work Kolhe et al [13] synthesized a fourth-generation PAMAM (PAMAM-OH) dendrimer covalently linked to ibuprofen using dicyclohexylcarbodiimide (DCC) as a coupling agent. A high payload nanocarrier was obtained; [49] molecules of ibuprofen were covalently conjugated to one molecule of PAMAM-OH (G4) dendrimer. FITC-labeled dendrimer–drug conjugate nanoparticles internalization was evaluated in vitro once using A549 cells. The pharmacological activity of the dendrimer–ibuprofen conjugate was compared to pure ibuprofen at various time points by measuring the suppression of prostaglandin E2. Results demonstrated the high internalization efficiency of the FITC-labeled dendrimer–drug conjugate and superior therapeutic activity due to faster prostaglandin E2 suppression. Thus, the results suggest that the dendrimer–ibuprofen conjugate improve the drug efficacy by enhanced cellular delivery, and may produce a rapid pharmacological response.

3. Dendrimers in Nasal Drug Delivery

Developing a noninvasive and safe alternative drug administration to substitute i.v. or i.t. administration is highly preferred because of their associated poor patient compliance [50]. Transmucosal routes of drug delivery (i.e., the mucosal linings of the nasal, rectal, vagina, ocular and oral cavity) offer distinct advantages over peroral administration for systemic drug delivery. These advantages includes possible bypass of the first pass effect, avoidance of pre-systemic elimination of gastro intestinal tract and depending on the particular drug.[51] Nasal administration offers an interesting alternative for achieving systemic drug effects to the parenteral route, which can be inconvenient or oral administration, which can result in unacceptably low bioavailabilities.[52]

Intranasal (IN) delivery is distinguished among the various strategies currently available for drug targeting. It is noninvasive and reduces the exposure of nontarget sites to therapeutic substances, thus increasing efficiency and safety of drug delivery. Drug molecules can be targeted to the brain from the nasal cavity through the olfactory nerve pathway and trigeminal nerve pathway, therein bypassing the BBB. In addition to this, drug molecules can be systemically absorbed from the nasal cavity and can subsequently permeate the BBB if the drug molecules have sufficient lipophilicity.[53] IN drug delivery is advantageous for water-soluble molecules, particularly biomolecules like proteins, which have poor bioavailability in brain tissues through oral or parenteral routes.[54,55] However, most small molecules that are used for therapy of CNS disorders have limited aqueous solubility. As such, they need to be formulated in nanoparticles or emulsions, solubilized using micellar solubilization or complexation with dextrans in order to be delivered to the brain via the IN route.[53,56] Although use of nanoparticles has been reported to improve the delivery of drugs to the brain via the IN route in several studies,[57-60] transport of large sized particles, especially larger than 200 nm, is not efficient due to poor paracellular and intracellular transport.[58]

Among various drug delivery systems, dendrimers due to their small size (less than 10 nm) are likely to have more efficient paracellular and transcellular transport across the BBB, [58] which makes them ideal carriers for targeting water insoluble drugs to the brain via IN administration. Dendrimers are discrete nanostructures/nanoparticles which are synthesized beginning with a core, and grown in concentric layers to produce stepwise increases in size.

Dendrimers have been reported to enhance the aqueous solubility of drugs by forming a complex with them[61] which would provide a high concentration of diffusible drug at the nasal area. The dendrimers can be selected so as to a have positive charge on the surface that would also lead to increased transport of drugs to the brain as positively charged nanoparticles have been reported to have increased association with mucus as well as greater cell uptake. [58]

In Nasal drug delivery Dendrimers can offer many advantages over conventional drug delivery. They:

- *Enhance the aqueous solubility of drugs*
- *Increased transport of drugs to the brain*
- *Have more efficient paracellular and transcellular transport across the BBB, which makes them ideal carriers for targeting water insoluble drugs to the brain*

Intranasal delivery of the water-soluble radiolabeled siRNA−dendrimer complexes (dendriplexes) incorporated into in situ forming mucoadhesive gels shows increased radioactivity in the brain as compared to that obtained following IN delivery of si RNA incorporated in the similar mucoadhesive gels.[62] However, to date there is no report on using dendrimers for targeting water-insoluble drugs to the brain via intranasal administration and how this route of administration compares with intraperitoneal administration of the same formulations.

Polyamidoamine (PAMAM) dendrimer has draws attention for nose-to-brain targeting. These dendrimers are repetitive branches that grow from a core. Many versatile molecules can be attached to their surface. Kim et al. connected an arginine onto the surface of a PAMAM dendrimer [63]. This resulted in nanoparticles with a size of 188.7 ± 1.9 nm and a charge of +22.3 mV. Small interference RNA (siRNA) targeting against the high mobility group box 1 protein (HMGB1) was electrostatically attached onto the nanoparticles. HMGB1 is released by dying cells and acts as a danger signal, thereby aggravating the damage of a stroke or other neurotoxic insults. Upon intranasal administration, they observed a wide distribution of the construct into the brain, including the hypothalamus, the amygdala, the cerebral cortex, and the striatum. Moreover, the localization of the PAMAM dendrimer and the siRNA was associated with an efficient knock-down of the protein of interest: HMGB1. When a stroke was induced into animals, the group that received the intranasal administration of the construct had a remarkably decreased infarction volume.

The potential of mucoadhesive gel of dendrimers formulations for nose to brain delivery was also displayed by Perez et al. [64]. They coupled radioactive siRNA to PAMAM dendrimers to form dendriplexes, and formulated these particles into mucoadhesive gels containing either 1% (w/w) chitosan or 0.25% (w/w) carbopol 974P NFTM. These gels were prepared by blending the chitosan or carbopol with 23% (w/w) of thermosensible poloxamer to obtain in-situ gelation. Such a thermosetting gel has a phase transition below the temperature in the nasal cavity (32 °C to 35 °C) and above room temperature. Therefore it can be administered as a liquid. Different concentrations of the different gels were tested and no toxicity was observed. Two intranasal doses were necessary to achieve higher brain concentrations of radioactivity than achieved by intravenous administration of dendriplexes or intranasal administration of naked siRNA.

4. Dendrimers in Gene Delivery

The ability to transfer genetic material efficiently, into the nucleus and cytoplasm of eukaryotic cells may allow treatment of a variety of genetic disorders. There are so many vectors and physical methods are reported for in vivo Gene delivery.

Commonly two approaches viz. viral and non‑viral based are being used to facilitate the gene delivery to target cells. Although viral carriers (Synthetic DNA delivery systems) can achieve rapid transfection, but low efficiency, immunological and oncologic adverse effects associated with these vectors has remained a topic of concern[65-67].

Non‑viral gene delivery vectors offer the usage of natural/synthetic molecules or physical forces to transfer genetic material to targeted cells. Several advantages

such as ease of fabrication, targeting ability, potential for repeat administration and low immune response have led to the usage of non‑viral vectors preferably for gene therapy.

Dendrimers are one of the most useful non-viral gene delivery systems and play a significant role in the development of non-viral vectors for gene delivery due to their ability to transfect cells without inducing toxicity, the high charge density and tunable surface functional groups, thus allowing optimal condensation and formation of nanostructures with DNA, the so-called "dendriplexes".

Many factors affect the efficiency of non-viral gene delivery systems. For successful gene therapy the genetic material should be permanently integrated and expressed by cells. In this context, Galetti et al.[68] demonstrated that antisense oligonucleotides (ONs, gene-specific sequences of nucleic acids with 15–25 bases) directed to LMP1 mRNA, effectively suppressed LMP1 gene expression, which plays a key role for growth transformation and immortalization of B lymphocytes. The efficiency of three cationic carriers on the delivery of anti-LMP1-ON to their site of action in Epstein Barr virus (EBV)-infected B lymphocytes was investigated. Results showed that liposomes, dendrimers or transferrin-PLL-conjugated ON were internalized by the cells at an extent several fold higher than that of the naked oligomers. Using Superfect®, a dendrimeric polycation with terminal amine groups, a higher intracellular concentration of ON was obtained as observed by both cytofluorimetric and confocal microscopy analyses. However, there was some evidence of toxicity induced by the positively charged dendrimers on the lymphocytes' membranes, and the lack of intracellular mRNA-ON duplex formation and of LMP1 mRNA degradation indicated a failure of this carrier.

Among various commercially available dendrimers, PAMAM dendrimers have received the most attention as potential non‑viral gene delivery agents due to their cationic nature which enables deoxyribonucleic acid (DNA) binding at physiological pH.[69]

Pandita et al. prepared dendrimer based gene delivery vectors taking advantage of the cationic nature and the "proton‑sponge" effect of these dendrimers. Arginine‑glycine‑aspartic (RGD) nanoclusters were formed by conjugation of G5 and G6 PAMAM dendrimers with a varying number of peptides containing the RGD motif, in view of its targeting capabilities. Authors reported that the system wherein G6 PAMAM dendrimer was conjugated to eight peptide arms enhanced the gene expression in mesenchymal stem cells and presented a 2‑fold higher bone morphogenetic protein‑2 expression in comparison to the G6 native dendrimer.[70] Various published literature suggests that functionalized dendrimers are much less toxic than the native dendrimers. Same group synthesized a new family of gene delivery vectors consisting of G5 PAMAM dendrimer core randomly linked to hydrophobic chains (with varying chain length and numbers). In vitro studies revealed a remarkable capacity of these vectors for internalizing pDNA with very low levels of cytotoxicity, being this effect positively correlated with the CH2 content present in the hydrophobic moiety. The results demonstrated that vectors containing the smallest hydrophobic chains showed the higher gene expression efficiency.[71] Further, functionalized PAMAM dendrimers exhibited low cytotoxicity and receptor‑mediated gene delivery into mesenchymal stem cells and transfection efficiencies superior to those presented by native dendrimers and by partially degraded dendrimers.[72]

The protection of DNA from in vivo degradation by the vectors is another key feature for success in gene delivery. Diaz-Mochon et al. [73] showed that a hybrid combination of PAMAM and peptide dendrimers, the so-called peptoid dendrimers, were able to transfect cells with higher efficiency than the PAMAM counterpart, and were nontoxic. In part, this work supported previous findings which demonstrated that combination of primary and secondary amines generates a "proton sponge" effect, which can facilitate theDNAtransfection process, by promoting the release of the plasmid from the cytoplasmic lysosome. Thus, efficiency of dendrimer/DNA complexes may be favored by prolonging the release of plasmid.

As aforementioned, cationic PAMAM dendrimers have proved to efficiently mediate transfection of DNA into a variety of mammalian cells, in vitro. However, as highlighted, the major drawback of high-generation cationic dendrimers is their associated cytotoxicity. Anionic dendrimers, on the other hand, have shown no cytotoxic effect on cells over a broad range of concentrations. Hussain et al.[74] have reported the successful use of ONs conjugated with pentaerythritol-based anionic dendrimers in inhibiting cancer-cell growth. In vitro studies using cancer cells showed that ONs-dendrimer conjugates enhance the cellular uptake, up to four times as compared to that for naked ONs. These data clearly demonstrated that anionic ONs-dendrimer conjugates may represent attractive alternatives to cationic non-viral vectors for the delivery of gene silencing ONs. However, it is not known whether the system may facilitate the delivery of duplex siRNA for gene silencing by RNA interference.

Vincent et al.[75] investigated the efficacy in cancer therapy of non-viral gene transfer using the anti-angiogenic angiostatin (Kringle 1–3) and tissue inhibitors of metalloproteinases (TIMP) genes. This study revealed that it was possible to inhibit tumor growth and angiogenesis by using PAMAM dendrimers-like superfectant associated with 36-mer anionic oligomers (ON36) for delivering angiostatin and (TIMP)-2 genes.

Luo et al.[76] revealed the low cytotoxicity of PEG-modified PAMAM and their efficiency on the DNA delivery. These systems were obtained using low generation dendrimers with PEG chains, which mimics the fractured high-generation dendrimers. In fact, the proposed molecules showed a 20-fold increase in

transfection efficiency as compared to that of partially degraded dendrimer controls.

5. Dendrimers in Vaccines Delivery

Most low molecular weight substances are not immunogenic; consequently, when it is desired to raise antibodies against small molecules, they must be conjugated to a macromolecule. In the past, natural proteins have commonly been used as carriers to generate antibodies to small molecules; now an alternative strategy using dendrimers has been demonstrated. In particular, unmodified PAMAM dendrimers that fail to elicit an antibody response on their own become haptenized upon protein conjugation and generate a dendrimer-dependent antigenic response [77-78].

Dendrimers have optimal characteristics to fill the need for efficient immunostimulating compounds (adjuvants) that can increase the efficiency of vaccines. Also dendrimers can provide molecularly defined multivalent scaffolds to produce highly defined conjugates with small molecule immunostimulators and/or antigens [79]. These molecules are ideal carriers of small antigens, making it possible to prepare multimeric antigenic conjugates with well-defined molecular properties for human uses and they do not induce adverse host responses, including immune and/or inflammatory reactions upon administration. The interest has focused on one specific class of dendrimers, namely, the peptide dendrons described in 2005 by Crespo et al. [80]

The basic structure described by Tam [81] is a dendron constructed solely from lysine, taking advantage of the two amino groups (R and ε) that are present in each lysine molecule and act as branching points for logarithmic growth. A two-layer dendron thus has four free amino groups and a three-layer dendron has eight, equally divided between Rand ε-amino groups. Tam coined the name "major antigenic peptide" (MAP) for such a structure derived from molecules of interest, not only limited to peptides, but also any small molecule that could bind covalently to the terminal amino groups of the MAP "core" dendron.

6. Dendrimers in Ocular Drug Delivery

The majorities of topically applied ocular drug-delivery systems are formulated either as solutions, ointments, or suspensions and suffer from various disadvantages such as quick elimination from the precorneal region, poor bioavailability, or failure to deliver the drug in a sustained fashion. The main challenge in ocular drug delivery is to increase the drug bioavailability and prolong the residence time of the drug on the cornea, conjunctival, and corneal epithelia. 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, and biodegradable. The viscosity of the final product should be optimized so that the dosage form does not run out of the eye. Dendrimers provide solutions to some complex delivery problems for ocular drug delivery.

Several nanotechnology based carrier systems are being developed and studied at large such as nanoparticles, liposomes, nanomicelles, nanosuspensions and dendrimers. Few of these are commercially manufactured at large scale and are applied clinically. However, there is still need of developing a carrier system which could reach targeted ocular tissue, including back of the eye tissues, post non-invasive mode of drug administration.

Tremendous efforts are being put into ocular research toward the development of safe and patient compliant novel drug delivery strategies.The unique nanostructured architecture of dendrimers has been studied to examine their role in delivery of therapeutics and imaging agents. Dendrimers can increase drug's water solubility, bioavailability, and biocompatibility and can be applied for different routes of drug administration successfully.

The first studies on dendrimers for ocular gene delivery were conducted in 1999. Chaum *et al* used the activated starburst PAMAM dendrimer SuperFect (Qiagen) as one of the transfection agents. The dendrimer consistently yielded the highest gene transfer efficiency into primary human RPE cells, with the DNA concentration and DNA: dendrimer concentration playing a major role in the transfection efficiency[82].

Other research efforts in dendrimers for ocular drug delivery include PAMAM dendrimers that were studied by Vandamme and Brobeck as ophthalmic vehicles for controlled delivery of pilocarpine and tropicamide to the eye [83].

PAMAM dendrimers with carboxylic or hydroxyl surface groups, have been reported in improving residence time and enhancing bioavailability of pilocarpine in the eye. [84] In the New Zealand albino rabbit model, the residence time of pilocarpine in the eye was increased by using dendrimers with carboxylic or hydroxyl surface groups. These surface-modified dendrimers were predicted to enhance pilocarpine bioavailability.

Shaunak et al. have synthesized water soluble conjugates of D(+)-glucosamine and D(+)-glucosamine 6-sulfate with anionic PAMAM (G3.5) dendrimers to obtain synergistic "immunomodulatory and antiangiogenic effect. [85]. These glucosamine and glucosamine 6-sulfate dendrimers were studied in a rabbit model of scar tissue formation after glaucoma filtration surgery. These unique polymeric macromolecules increased the long-term success of the surgery from 30% to 80% when used together. Furthermore, neither microbial infections nor clinical, biochemical, or hematological toxicity was observed in all animals

In another study, lipophilic amino-acid dendrimers were used to study the long-term effect of use of dendrimer for delivery of an antivascular endothelial growth factor (VEGF) oligonucleotide (ODN-1) to the eye of rats with

 ISSN: 2250-1177 CODEN (USA): JDDTAO

the aim of inhibiting laser-induced choroidal neovascularization (CNV). It was shown that dendrimer containing ODN-1 showed significantly greater inhibition of CNV over a 4–6 month period compared with ODN-1 alone [86]. Immunohistochemistry of the eye tissue after long-term treatment with dendrimers was conducted to determine if an immune response was generated after use of the dendrimer as a drug conjugate for treating eye diseases. It was determined that there was no significant increase in inflammatory response, proving that dendrimers could be used as a viable option for delivery of oligonucleotide to the eye for treating angiogenic eye diseases without concern of generating unwanted biological response.

In a recent study, Puerarin–dendrimer complexes were prepared using PAMAM dendrimers (G3.5, G4, G4.5, and G5) and their physicochemical properties, *in vitro* release, corneal permeation, and ocular residence times were determined. Valia-Chien evaluated the corneal permeation and ocular residence time in rabbits using diffusion cells with excised corneas. It was reported that puerarin-dendrimer complexes exhibited longer residence time in rabbit eyes than puerarineyedrops, withoutdamage tothe corneal epithelium or endothelium. Also results of the *in vitro* release studies showed that puerarin release was much more slower from complexes than the free puerarin in PBS. However, corneal permeation studies suggested that there was no significant difference between puerarin-dendrimer complexes and puerarin eye drops on drug permeability coefficient [87].

A PAMAM dendrimer hydrogel has been developed by Holden and coworkers that is made from “ultraviolet-cured PAMAMdendrimer” linkedwith PEG-acrylate chains for the delivery of two antiglaucoma drugs which were brimonidine (0.1% w/v) and timolol maleate (0.5% w/v). Dendrimeric hydrogel was obtained by crosslinking of the reactive acrylate groups, triggered by UV light. It was reported that the dendrimeric hydrogel was mucoadhesive and nontoxic to epithelial cells of human cornea.Higher uptake from“human corneal epithelial cells” and significantly enhanced “bovine corneal transport” were reported for both drugs, compared to the eye drops. The higher uptake in the dendrimeric hydrogel formulations explained the temporary decomposition of the corneal epithelial tight junctions [88].

In conclusion, they are best suitable drug delivery systems with improved bioavailability of drugs, increased retention time, reduced side effects, cellular targeting, better patient compliance, and providing extended therapeutic effects in case of ocular delivery

7. Dendrimers in Topical and Transdermal delivery

The ability of transdermal drug delivery systems (TDDS) to deliver and maintain a constant therapeutic concentration of drug offers a significant potential for safe administration of therapeutic agents. TDDS can provide a steady drug blood concentration and thus avoid peaks and valleys in the drug plasma levels, which occur with traditional dosing, such as oral administration and intravenous administration.

Also, sustained/prolonged delivery of therapeutic agents in TDDS can simplify the dosing schedule and minimize the pain during traditional drug administration.[89] Besides, TDDS can improve patient compliance and eliminate the hepatic first-pass effect and chemical degradation in the gastrointestinal tract.109 Additionally, patients can choose elsewhere on the skin to conduct the TDDS according to their need because skin is the largest and most easily accessible organ in the body.

However, transdermal delivery of drugs is limited due to the slow rate of transdermal delivery. The outer layer of the skin which is served as the first line of defense, is composed of closely packed dead cells formed by epidermal differentiation and cornification.[90]

The most common method to improve drug penetration through the skin is to use transdermal enhancers. Various transdermal enhancers were employed in the past two decades. Chemical transdermal enhancers, such as organic solvents, are effective because they can directly react with the skin, and thus transiently increase their permeability but induce immune responses in the skin. Therefore, polymeric enhancers with hydrophilic and hydrophobic properties have attracted increasing interest. PAMAM dendrimers can improve either the water-solubility or stability of hydrophobic drugs. These materials with hydrophilic outer shells and hydrophobic interiors, which accord with structural requirement of polymeric transdermal enhancers, are expected to act as effective penetration enhancers.

Wang et al. reported the utilization of polyhydroxyalkanoate (PHA) and G3 PAMAM dendrimer as a novel TDDS. PHA used in this experiment was composed of 3-hydroxyhexanoic acid (8%) and 3-hydroxyoctanoic acid (92%)[90]. Before the in vitro permeation experiments performed on snake skins, PHA and the model drug (tamsulosin hydrochloride) were mixed together and laid on the transdermal delivery patches. In order to determine the effect of the PAMAM dendrimer on in vitro penetration efficiency of tamsulosin hydrochloride, they pretreated the snake skin with PAMAM dendrimer solution for 24 h. However, no significant enhancement of permeation amount of the model drug was found. When PAMAM dendrimer was coadministrated with the PHA matrix, the penetration amount of tamsulosin in the dendrimer-containing PHA matrix was 24.0 mg/cm2/day while that in the dendrimer-lacking PHA matrix was 15.7 mg/cm2/ day (the required amount of this drug in clinical trails is 20 mg/cm2/day). Thus, the authors concluded that PHA-dendrimer matrix reached the clinical aim and could be developed as a useful delivery system for clinical TDDS.

Dendrimers have found recent applications in novel topical and transdermal delivery systems, providing benefits such as improved drug solubilization, controlled release, and drug-polymer conjugates (pro-drugs). The viscosity-generation-number property of a dendrimer

solution allows for ease of handling of highly concentrated dendrimer formulations for these applications. Dendrimers have been shown to be useful as transdermal and topical drug delivery systems for nonsteroidal anti-inflammatory drugs (NSAIDs), antiviral, antimicrobial, anticancer, or antihypertensive drugs. PAMAM dendrimers have been studied as carrier transdermal systems for the model NSAIDs: ketoprofen and diflunisal [91]. It was found that the PAMAM dendrimer-drug formulations showed increased transdermal drug delivery compared with formulations lacking dendrimers. *In vivo* studies in mice showed prolonged pharmacodynamic responses and 2.73-fold higher bioavailability over 24 h for certain dendrimer-containing drug solutions.

In another study, transport of indomethacin through intact skin was enhanced *in vitro* and *in vivo* [92]. The bioavailability of indomethacin was increased by using G4-PAMAM dendrimers with terminal amino groups. There have also been studies where dendrimers failed to show enhancement in drug transport through intact skin. It is well known that the molecular diffusion through intact skin is related to the molecular weight of the permeant molecule. Because of their high molecular weights, dendrimers generally have low diffusion coefficients. Diffusion through skin is more favorable for molecules that have solubility in lipids as well as in water. It could be possible to synthesize dendrimers with appropriate physical-chemical properties to facilitate drug transport through intact skin. Dendrimers with such favorable physicochemical properties could enhance transdermal transport of drugs by this mechanism. More research is warranted in this area to understand the structural-activity relationship of dendrimers in relation to skin transport.

In contrast to transdermal delivery, the use of dendrimers for topical delivery to the skin has shown to be more promising. Two different kinds of dendrimers were shown to have antiviral activity *in vitro* when the dendrimers were added to the cells before being challenged with the viruses. The dendrimers studied were either PAMAM or polylysine dendrimers. In contrast, dendrimers added to the cells after they were challenged with the virus showed no antiviral activity. The study was carried out in an *in vitro* assay to determine dendrimer activity against herpes simplex virus (HSV) types 1 and 2. When tested in human foreskin fibroblast cells, both PAMAM and polylysine dendrimers showed activity against the virus. This study suggested that dendrimers could potentially be used as topical microbicides to be applied to the vaginal or rectal mucosa to protect against sexually transmitted diseases such as HIV or genital herpes. When tested against genital HSV infection in mice, two of the compounds showed significant reduction in infection rates when applied prior to intravaginal challenge.

Dendrimers are able to improve drug properties such as solubility and plasma circulation time via transdermal formulations and to deliver drugs efficiently due to its highly water soluble and biocompatible nature. For example improving the drug permeation through the skin when PAMAM dendrimer complex with NSAIDs like Ketoprofen, Diflunisal and enhanced bioavailability of PAMAM dendrimers by using indomethacin as the model drug in transdermal drug application[91,92].

8. Dendrimers in Pulmonary drug delivery

The lung is an attractive target for drug delivery due to noninvasive systemic delivery via inhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action for the treatment of respiratory diseases, and the availability of a huge surface area for local drug action and systemic absorption of drug.

Nanocarrier systems like dendrimers in pulmonary drug delivery offer many advantages. These advantages include the following:

1) The potential to achieve relatively uniform distribution of drug dose among the alveoli;
2) An achievement of enhanced solubility of the drug than its own aqueous solubility;
3) The sustained-release of drug which consequently reduces the dosing frequency;
4) Suitability for delivery of macromolecules;
5) Decreased incidence of side effects;
6) Improved patient compliance; and
7) The potential of drug internalization by cells.

Kukowska-Latallo and colleagues[93] investigated the ability of polyamido amine (PAMAM) dendritic polymers (dendrimers) to augment plasmid DNA gene transfer *in vivo* and evaluates the targeting of the lung by alternative routes of administration. They suggested that vascular administration seemed to achieve expression in the lung parenchyma, mainly within the alveoli, while endobronchial administration primarily targeted bronchial epithelium, indicating that each delivery route requires different vectors to achieve optimal transgene expression that each approach appears to target different cells within the lung.

Rudolph and colleagues[94] compared the properties of branched polyethylenimine (PEI) 25 kDa and fractured PAMAM dendrimers for topical gene transfer to the airways *in vivo*. Their results demonstrated that gene transfer mediated by PEI under optimal conditions was two orders of magnitude higher compared to fractured dendrimers. Therefore, branched PEI 25 kDa was superior to fractured dendrimers for gene delivery to the airways.

Polyamidoamine dendrimers with positive charge have shown the enhanced bioavailability in pulmonary delivery of low-molecular weight heparin (a negatively charged oligosaccharide) to treat vascular thromboembolism. In this formulation, dendrimer-drug complex was formed [95]. Further studies showed that heparin encapsulated in pegylated dendrimers has a longer circulating half-time and increased pulmonary absorption [96].

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 intervals [97]

In addition to dendrimers, cationic liposomes were used as carriers for heparin and showed enhanced pulmonary absorption. These cationic liposomes were prepared by conventional methods, i.e. lipid dispersion, solvent evaporation and extrusion [98].

9. Dendrimer as Solubility enhancer

With the discovery of new drug molecules today low solubility is the main hurdle to be overcome[99]. Approximately 40% of newly developed drugs are rejected by the pharmaceutical industry and will never benefit a patient because of low water solubility. Given the growing impact and need for drug delivery, a thorough understanding of delivery technologies that enhance the bioavailability of drugs is important. The high level of control over the dendritic architecture (size, branching density, surface functionality) makes dendrimers ideal excipients for enhanced solubility of poorly water-soluble drugs. Many commercial small-molecule drugs with anticancer, anti-inflammatory and antimicrobial activity have been formulated successfully with dendrimers, such as poly (amidoamine) (PAMAM), poly(propylene imine) (PPI or DAB) and poly(etherhydroxylamine) (PEHAM). Some dendrimers themselves show pharmaceutical activity in these three areas, providing the opportunity for combination therapy in which the dendrimers serve as the drug carrier and simultaneously as an active part of the therapy. Dendrimers are unimolecular micellar nature, due to have hydrophilic exteriors and hydro-philic interiors and form covalent as well as non-covalent complexes with drug molecules and hydrophobes, and enhance its solubilisation behaviour[100].

10. Dendrimers in Cellular delivery

Medical therapies have become more tailored to specific diseases and patients in recent years. Most pharmaceutical agents have primary targets within cells and tissues; ideally, these agents may be preferentially delivered to these sites of action within the cell. Selective subcellular delivery is likely to have greater therapeutic benefits. Cytosolic delivery, for instance, is desirable for drugs that undergo extensive exportation from the cell via efflux transporters such as multi-drug resistance proteins and P-glycoproteins. These efflux mechanisms continuously reduce therapeutic intracellular drug concentrations.

Appropriate surface-functionalized dendrimers can enter certain cells remarkably well and, hence, are under active investigation aspotential drug delivery and gene-transfection agents. The objective is to deliver a therapeutic drug or gene payload to a specific intracellular site for desired local action. The intracellular delivery of dendrimer nanocarriers involves both extracellular drug release at the interstitium (tissue site) and intracellular delivery upon internalization. It is essential that the dendrimer nanocarrier loaded with a drug or gene is not cleared too quickly from circulation. The design of a suitable delivery system requires elimination or minimization of all nonspecific interactions that might occur between the dendrimer vector and the environment of the systemic compartment. A primary function of the carrier is to mask all unwanted interactions between the drug and the environment until the drug is released from the carrier at the target site [19].

PAMAM dendrimers with lauryl chains to reduce toxicity and enhance cellular uptake, for example Dendrimer ibuprofen complexes entered the cells rapidly compared with pure drug (1 hr versus>3 hr), suggesting that dendrimers can efficiently carry the complexes drug inside cells[101].

11. Dendrimers as Bio mimetic artificial proteins

Dendrimers are often referred to as “artificial proteins” due to their dimensional length scaling, narrow size distribution, and other bio mimetic properties. For examples PAMAM family, they closely match the sizes and contours of many important proteins and bio assemblies like insulin (3 nm), cytochrome C (4 nm), and haemoglobin (5.5 nm) are approximately the same size and shape as ammonia-core PAMAM dendrimers generations 3, 4 and 5, respectively. Generation 2 dendrimer matches the width (2.4 nm) of DNA duplexes (form stable complexes with histone clusters to condense and store DNAwithin the nucleosome of cells.) and generations 5 and 6 PAMAM dendrimers have diameters approximately equivalent to the thickness of lipid bilayer membranes (~5.5 nm) of biological cells[102, 103].

12. Dendrimers as Nano-Drugs

A number of nano-based systems allow delivery of insoluble drugs, allowing the use of previously rejected drugs or drugs which are difficult to administer[104]. The key useful character of dendrimers is the branches which can provide vast amounts of surface area for drugs and targeting molecules. Meanwhile, the surface functionalities, interior branching, and chemical composition of the core play a significant role in reactivating the macromolecule. Dendrimer is one of the most elegant nanotechnology platform s for targeted drug delivery[105] Conjugated with biotin as the targeting moiety; the in vitro targeting ability of partially acetylated generation 5 polyamidoamine (PAMAM) dendrimer (Ac-G5) in HeLa cells was assessed. The multi-functional conjugate Ac-G5-biotin-FITC (fluorescein isothiocyanate) showed much higher cellular uptake than the conjugate without biotin. The energy dependent uptake process can be blocked effectively by biotin polymer conjugates, exhibiting an expected dose response curve.

Dendrimers as Nano-Drugs, useful as antiviral drugs against the herpes simplex virus can potentially prevent/reduce transmission of HIV and other sexually transmitted diseases (STDs) when Poly (lysine) dendrimers modified with sulfonated naphthyl groups. Show potent antibacterial biocides against Gram positive and Gram negative bacteria when PPI dendrimers with tertiary alkyl ammonium groups attached to the surface and Chitosan– dendrimer hybrids have been found to be useful as antibacterial agents, carriers in drug delivery systems, and in other biomedical applications[84, 106].

13. Dendrimers in Site Specific Drug Delivery

Effective targeted drug delivery systems have been a dream for a long time, but it has been largely frustrated by the complex chemistry that is involved in the development of new systems[107]. The concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localised on the targeted site. Hence, surrounding tissues are not affected by the drug[108].

The targeted delivery of chemotherapeutics is essential to reduce the side effects significantly associated with conventional therapy, where healthy tissues such as liver, spleen, kidneys and bone marrow can accumulate the toxic levels of drug. The site specific delivery of the drug could be achieved by surface modification of dendrimers employing various targeting moieties such as folic acid (FA), peptides, monoclonal antibodies and sugar groups.[109]

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 usually achieved by increasing the hydrodynamic radius of the dendrimer though PEGylation, leading to the accumulation of dendrimer in tumor tissue via the enhanced permeability retention (EPR) effect. The EPR effect is a result of tumorinduced 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 [110]. Specific or active targeting relies on the conjugation of one or more targeting moieties to the dendrimer to facilitate cell-receptor-mediated interactions.

Several successful active and passive targeting attempts were accomplished by engineering the branching units and surface groups of dendrimers. Patri et al. conjugated FA to G5 PAMAM dendrimer for the targeted delivery of methotrexate and observed receptor mediated drug delivery that demonstrated high specificity for KB cells overexpressing folate receptors and showed slower drug release.[35] The authors further conjugated the PhiPhiLux G1 D2, an apoptotic sensor to FA attached PAMAM dendrimers which showed 5 fold enhanced fluorescence, attributed to successful delivery of drug with cell‑killing efficacy.[111]

pH and temperature-activated polymers are known to be successful drug delivery systems. Photochemical internalization (PCI) can facilitate site-specific delivery, e.g., escape of the macromolecules from endocytic vesicles into the cytosol. Lai et al. [112] conjugated doxorubicin (Dox) to PAMAM dendrimers via pH-sensitive and insensitive linkers, acid-labile hydrazone linkages (PAMAMhyd- Dox) and amide (PAMAM-amide-Dox), respectively.

They combined doxorubicin-dendrimers with different PCI strategies to evaluate the cytotoxic effects. Results showed that both PCI strategies promoted the PAMAM-amide-Dox cytosolic distribution, but significantly enhanced the cytotoxicity of free Dox on human gingival cancer (Ca9-22) cells at higher concentrations. The authors failed to develop a multi-modality cancer treatment, but their data provided insights on possible research directions, namely the need to exploit spacers other than amide-linkage in drug-polymer complexes.

Wiwattanapatapee et al. [113] investigated the use of dendrimers for colon-specific drug delivery applications. In their studies, 5-aminosalicylic acid (5-ASA) was bound to the water-soluble dendrimer using different spacers containing azo-bond (e.g., p-aminobenzoic acid, PABA and p-aminohippuric acid, PAH). PAH provide the polymer conjugates a higher loading capacity (3 times) for 5-ASA as compared to that of dendrimer conjugates with PABA as the spacer. In vitro studies of rats with cecal content were carried out to investigate drug release from dendrimer conjugates. The release of 5-ASA from both conjugates was significantly slower as compared to that of sulfasalazine (SA), a commercial prodrug. Moreover, the conjugate with PAH linker showed significantly higher amount of initial drug release than the conjugate with the PABA linker. As a consequence, the amount of drug released from PAMAM-PAH-SA was significantly higher than that of PAMAM-PABA-SA conjugate. This study nicely illustrated the potential use of PAMAM dendrimer for colon-specific drug delivery, and the important role of the spacers for the optimization of drug release.

CD derivatives bearing peptides may be useful as carriers for transporting drugs to biological targets containing specific peptide receptors. Thus, peptide biorecognizability together with the CD host–guest complexation properties makes such systems suitable templates for the application in site-specific drug delivery. Much effort has beenmadeto complex low generation dendrimers with other polymers. For example, Dodziuk et al. [114] reported attempts to complex a first-generation dendrimer having four branches with α, β or γ cyclodextrins, found to be unsuccessful in their subsequentNMRstudies. Muhanna et al. [115] reported a different strategy, with the synthesis of tetradecavalent

amino acid and peptide dendrimers based on a β -CD core. These were found to have great potential for application in MAP concept as a means to increase the peptide–receptor binding, and hence improve the site specificity of the drug delivery system.

14. Dendrimers in Intravenous Drug Delivery:

The intravenous route is the rapidest and simplest method for delivering a drug into the systemic circulation.[116] However, poor water solubility of many drugs, especially anti-cancer drugs, limits the application of intravenous administration route in clinical trails. Intravenous administration of these drugs results in several side effects, such as hemolysis and phlebitis.[117]

Much effort has been made to develop new formulations that are suitable for the intravenous route, among which dendrimer-drug formulation is attracting increasing interests as one of the emerging delivery systems. Their biodistribution in the body and toxicity or immunogenicity must be consider before the proposed application of dendrimers in the intravenous route.[118], [119]

Meanwhile, in vivo biodistribution of dendrimers after intravenous administration has also been intensively studied. Kukowska-Latallo et al.[120] investigated the biodistribution of tritium labeled G5 PAMAMdendrimers after intravenous administration and found these materials were cleared rapidly from the blood via the kidney during the first day postinjection.

Bhadra et al. used G4 PAMAMdendrimer and PEG-5000 to synthesize PEGylated dendrimers, which were applied as potential drug carriers of 5-fluorouracil (5-FU, an anti-cancer drug). After intravenous administration of different formulations of 5-FU (equivalent 5-FU in each formulation) to rats through the caudal vein, the maximum drug concentration (Cmax) from free drugs, nonPEGylated dendrimers, and PEGylated dendrimers, was 200–220, 21–23, and 6-7 mg/mL, respectively[121]. The blood level of 5-FU in PEGylated dendrimers formulations was still detectable up to 12 h after the drug was administrated. In a previous research, Bhadra et al investigated the behavior of G4 PAMAM dendrimer/indomethacin complex (indomethacin, a nonsteroidal anti-inflammatory drug) after intravenous administration.[122] They obtained enhanced effective indomethacin concentrations (2.29 times) in the inflamed regions with the dendrimer/indomethacin complex when compared to the free drug in arthritic rats. Although the lymphatic drainage existed, intravenous administration of the complex still prolonged the retention of the drug at the inflamed site.

FUTURE PROSPECTS

Dendrimeric polymers are very important and convenient for different types of drugs delivery. In order to be effective, dendrimer-based products should be based on scientific evidence for their usefulness and must be easier to translate from laboratory to the clinic, in other words be quality-controlable, cost-effective and sustainable.

Literature review of biomedical applications of the dendrimers clearly illustrate the potential of this new fourth architectural class of polymers and substantiate the high optimism for the future of dendrimers in drug delivery, diagnosis and therapy. Scientists have explored the use of dendrimers for various applications in oral, transdermal, ophthalmic, and gene delivery. Although dendrimer drug delivery requires attention to certain manufacturing and biological considerations to be successful.

Besides drug delivery, dendrimers have been found to have a great emphasis in drug delivery through nasal, CNS, transdermal, ocular, oral and I.V. route. The use of dendrimers in the clinic has still not reached the success of linear polymers and several applications remain to be explored for its industrial as well as biomedical applications. With improved synthesis, further understandings of their unique characteristics and recognition of new applications, dendrimers will become promising candidates for further exploitation in drug discovery and clinical applications. Boosting of commercial applications of dendrimer technology will provide strength for its usefulness in future.

Targeted delivery is still an active research area for the application of dendrimers in CNS drug delivery. Several current targets such as LDL receptors, insulin receptor, and transferrin receptor have been also found in other tissues, suggesting that they are not specific. More specific and efficient targets need to be identified to facilitate the development of safer and more effective dendrimer delivery systems for use in the CNS.

However, challenges still exist regarding the deeper toxicological studies, specific targeting, and noninvasive alternative drug administration methods. The ultimate goal of dendrimer-mediated drug-delivery systems is to engineer the dendrimers to be safe and to enable their longterm use without the accumulation of adverse effects.

CONCLUSION

Dendrimers can work as a useful tool for optimizing drug delivery of such problematic drugs. Although the application of dendrimers in the field of drug, gene, and vaccine delivery is in its infancy, dendrimers offer several attractive features, including the control one has over the primary nature of the system. They provide a platform for the attachment of drugs or genes and their release through several mechanisms.

The high level of control over the architecture of dendrimer, their shape, branching length and density, their surface functionality and interior void space (porosity) and so on makes dendrimer ideal carriers for the various applications like drug delivery, therapeutic and diagnostic agent. Poor solubility, bioavailability and permeability biocompatibility and toxicity can be overcome by use it.

PEGylation of the dendrimer surface can prolong its circulation time and reduce its toxicity. BBB- or CNS-targeting ligand modification of the dendrimer surface can improve the rate and duration of drug delivery to

brain tumor cells prior to the clearance of the remaining drug-delivery system.

Hopefully, this review of dendrimer-based medical applications clearly illustrates the potential application of this new 'fourth architectural class of polymers' and reaffirms an even higher level of optimism for the future role of dendrimers in the drug delivery. With continued research and development efforts, dendrimer is expected to have a tremendous impact on delivery of drugs in future.

DECLARATION OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper

Abbreviations: 2-AS, 12-(9-anthroyloxy) stearic acid; 2-D, two-dimensional; 3-D, three-dimensional; 3-TC, lamivudine; Ac, acetylated; AChE, acetylcholinesterase; AF, alexaFluor; AFM, atomic force microscopy; ATRP, atom transfer radical polymerization; b-FGF, basic fibroblast growth factor; BAPTA-AM, 1,2-bis-(o-aminophenoxy) ethane-N,N,N',N',-tetraacetic acid-acetoxymethyl ester; BMVEC, brain micro-vessel endothelial cells; BNTC, boron neutron capture therapy; CLB, chlorambucil; CED, convection enhanced delivery; CMCht, carboxymethylchitosan; CMCht/PAMAM, carboxymethylchitosan/poly (amidoamine); DCC, dicyclohexylcarbodiimide; Dex, dexamethasone; di-BOC, di-tert-butyl dicarbonate; DOTAP, N-(1-(2,3-dio-leoyloxy) propyl)-N,N,N-trimethyl ammonium methylsulfate; EBV, Epstein Barr virus; ECM, extracellular matrix; EGF, epidermal growth factor; EGFP, green fluorescent protein; ENFET, enzyme field-effect transistor; EPR, electron paramagnetic resonance; FA, folic acid; FITC, fluorescein isothiocyanate; G, generation number; GAGs, glycosaminoglycans; GLUT 1, glucose transporter; GOX, glucose oxidase; HA, Hyaluronic acid; HAp, hydroxyapatite; HAS, human serum albumin; HBP, hyperbranched polymer; HER-2, human growth receptor; HMGB1, high mobility group box 1 plasmid; HSGP, heparin or heparan sulfate proteoglycan; i.p., intraperitoneal; i.t., intratumoral; LCST, lower critical solution temperature; LH, light-harvesting; MA, methacrylate; MAPs, multiple antigen peptides; Man, mannose; Mn, number-average molecular weight; Mo/Mac, monocyte/macrophages; MPPI, poly(propyleneimine); MRI, magnetic resonance imaging; MS, multiple sclerosis; Mw,weight-average molecular weight,Mw/Mn polydispersity index; NaHA, sodium hyaluronate;NMR,nuclear magnetic resonance; OEI, oligoethylenimine; OG, oregon green; ONs, antisense oligonucleotides; PAMAM, poly(amidoamine); PAH, p-aminohippuric acid; PCI, photochemical-internalization; PDMA, poly(N,N-dimethylaminoethyl methacrylate); PEG-DA, poly(ethylene glycol)-dialdehyde; PEI, poly(ethyleneimine); PEPE, polyether-copolyester; PETIM, poly(propyl ether imine); PLGSA, poly(glycerol-succinic acid); PLL, poly-(l-lysine); PNIPAAM, poly(N-isopropylacrylamide); PP, primaquine phosphate; PPI, poly(propyleneimine); PrPSc, protease-resistant isoform of the prion protein; PSMA, prostrate specific membrane antigen; PTX, paclitaxel; RAFT, reversible addition-fragmentation transfer; RGD, Arg-Gly-Asp peptides; ROS, reactive oxygen species; SA, sulfasalazine; SEM, scanning electron microscopy; shRNA, small hairpin RNA; t-BOC, N-tert-butoxycarbonyl; TE, tissue engineering; ThT, thioflavin T; TIMP, tissue inhibitors of metalloproteinases; TMA-DPH, 1 (trimethylammoniumphenyl)-6-phenyl-1,3,5 hexatriene p-toluenesulfonate; VEGF, vascular endothelial growth factor.

How to cite this article:
Parajapati SK, Maurya SD, Das MK, Tilak VK, Verma KK, Dhakar Ram C, Potential application of dendrimers in drug delivery: A concise review and update, Journal of Drug Delivery & Therapeutics. 2016; 6(2):62-70

REFERENCES

1. Dwivedi DKr, Singh AK, Dendrimers: a novel carrier system for drug delivery, 2014; 4(5):1-6
2. D'Emanuele A, Jevprasesphant R, Penny J, Attwood D. The use of a dendrimer-propranolol prodrug to bypass efflux transporters and enhance oral bioavailability. J Control Release 2004; 95:5447–53.
3. Tomalia DA, Birth of a new macromolecular architecture: dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. Prog Polym Sci 2005;30:294–324.
4. Boas U, Jørn Bolstad Christensen, Heegaard PMH, "Dendrimers in medicine and biotechnology: new molecular tools", 2006, 62-70
5. Mishra Ina, Dendrimer: a novel drug delivery system, Journal of Drug Delivery & Therapeutics; 2011; 1(2):70-74
6. Allen TM, Cullis PR. Drug delivery systems: Entering the mainstream. Science 2004; 303:1818‐22.
7. Soto‐Castro D, Cruz‐Morales JA, Ramírez Apan MT, Guadarrama P. Solubilization and anticancer‐activity enhancement of Methotrexate by novel dendrimeric nanodevices synthesized in one‐step reaction. Bioorg Chem 2012;41‐2:13‐21.
8. Duncan R, Izzo L. Dendrimer biocompatibility and toxicity. Adv Drug Deliv Rev 2005; 57:2221-2237.
9. Patton DL, Cosgrove Sweeney YT, McCarthy TD, Hillier SL. Preclinical safety and efficacy assessments of dendrimer-based (SPL7013) microbicide gel formulations in a nonhuman primate model. Antimicrob Agents Chemother 2006; 50:1696-700.
10. Prajapati S., Maurya S., Das M., Tilak V., Verma K.K., Dhakar R.C., Dendrimers in drug delivery, diagnosis and therapy: basics and potential applications. Journal of Drug Delivery and Therapeutics, 2016; 6(1):67-92. Available at: <http://jddtonline.info/index.php/jddt/article/view/1190>. Date accessed: 25 Feb. 2016.
11. Hawker CJ and J.M. J. Fr´echet, "Preparation of polymers with controlled molecular architecture. A new convergent approach to dendriticmacromolecules," *Journal of the American Chemical Society*, 1990; vol. 112, no. 21, pp. 7638–7647.
12. Esfand R and Tomalia DA, "Poly (amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications," *Drug Discovery Today*, 2001; vol. 6, no. 8, pp. 427–436.
13. Kolhe P, Khandare J, Pillai O, Kannan S, Lieh-Lai M, and Kannan RM, "Preparation, cellular transport, and activity of polyamidoamine-based dendritic nanodevices with a high drug payload," *Biomaterials*, 2006; vol. 27, no. 4, pp. 660–669.
14. Khandare JJ, Jayant S, Singhet A al., "Dendrimer versus linear conjugate: influence of polymeric architecture on the delivery and anticancer effect of paclitaxel," *Bioconjugate Chemistry*, 2006; vol. 17, no. 6, pp. 1464–1472.
15. D'Emanuele A and Attwood D, "Dendrimer-drug interactions," *Advanced Drug Delivery Reviews*, 2005, vol. 57, no. 15, pp. 2147– 2162.
16. Gupta U, Agashe HB, Asthana A, and Jain NK, "Dendrimers: novel polymeric nanoarchitectures for solubility enhancement," *Biomacromolecules*, 2006; vol. 7, no. 3, pp. 649–658.
17. Aulenta F, Hayes W, and Rannard S, "Dendrimers: a new class of nanoscopic containers and delivery devices," *European Polymer Journal*, 2003; vol. 39, no. 9, pp. 1741–1771.

18. Gillies ER and Fr´echet JMJ, "Dendrimers and dendritic polymers in drug delivery," *Drug Discovery Today*, 2005; vol. 10, no. 1, pp. 35–43.
19. Menjoge AR, Kannan RM and Tomalia DA, Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications, Drug Discovery Today. 2010; 15(5/6):171-185.
20. Yang, H. and Lopina, S.T. Stealth dendrimers for antiarrhythmic quinidine delivery. J. Mater. Sci. Mater. Med. 2007, 18, 2061–2065.
21. Morgenroth F, Reuther E, Mullen K, Polyphenylene Dendrimers: From Three-Dimensional to Two-Dimensional Structures Angewandte Chemie, International Edition in English, 1997; 36 (6):631-634.
22. Nanjwade BK, Hiren M, Dendrimers: Emerging polymers for drug-delivery systems, Eur J Pharm Sci., 38 (3),2009, 185-196.
23. Sakthivel T, Florence AT. Dendrimers and dendrons: facets of pharmaceutical nanotechnology, Drug delivery technology, 2003; 73-78.
24. D' Emanuele A, R. Jevprasephant. The use of a dendrimer – propranolol prodrug to bypass efflux transporters and enhance oral bioavailability, Journal of controlled release, 2004; 95: 447-453.
25. Cloninger MJ. Biological applications of dendrimers. Curr Opin Chem Biol 2002; 6:742‐ 8.
26. Jang WD, Kamruzzaman Selim KM, Lee CH, Kang IK. Bioinspired application of dendrimers: From bio‐ mimicry to biomedical applications. Prog Polym Sci 2009; 34:1‐ 23.
27. Cheng Y, Wu Q, Li Y, Hu J, Xu T. New insights into the interactions between dendrimers and surfactants: 2. Design of new drug formulations based on dendrimer‐ surfactant aggregates. J Phys Chem B 2009; 113:8339-46.
28. Jansen JF, de Brabander‐ van den Berg EM, Meijer EW. Encapsulation of guest molecules into a dendritic box. Science 1994; 266:1226 9.
29. D'Emanuele A, Attwood D. Dendrimer‐ drug interactions. Adv Drug Deliv Rev 2005; 57:2147‐ 62.
30. Parekh Hejal B, Jivani Rishad, Jivani NP, Patel LD, Makwana Ami, Sameja Krunal, Novel insitu polymeric drug delivery system: a review, Journal of Drug Delivery and Therapeutics, 2012; 2(5):136-145
31. Panda Priyabrata, Mishra Sangeet Sarita, Pati Kanhu Charan, Nano-medicine: an emerging trend in molecular delivery, Journal of Drug Delivery and Therapeutics, 2014: Special Issue1: "Drug Delivery using Nanomedicine & Nanotechnology", 98-106.
32. Kaur H, Singh G, *In-vivo* methods to study uptake of nanoparticles into the brain, Journal of Drug Delivery and Therapeutics; 2013, 3(4):173-177
33. Yang H, "Nanoparticle-Mediated Brain-Specific Drug Delivery, Imaging and Diagnosis," *Pharmaceutical Re-search*, Vol. 27, No. 9, 2010, pp. 1759-1771. doi:10.1007/s11095-010-0141-7
34. Leyuan Xu, Hao Zhang, Yue Wu, Dendrimer Advances for the Central Nervous System Delivery of Therapeutics, ACS Chem. Neurosci. 2014; 5:2−13
35. Nowacek, A., and Gendelman, H. E. NanoART, neuroAIDS and CNS drug delivery. Nanomedicine. 2009; 4:557−574.
36. Wong, H. L., Wu, X. Y., and Bendayan, R. Nanotechnological advances for the delivery of CNS therapeutics. Adv. Drug Delivery Rev. 2012; 64, 686−700.
37. Kitchens KM, El-Sayed ME, Ghandehari H, "Transepithelial and Endothelial Transport of Poly(ami-doamine) Dendrimers," *Advanced Drug Delivery Reviews*, Vol. 57, No. 2005, pp. 2163-2176.
38. Heather A. Bullen, Ruth Hemmer, Anthony Haskamp, Chevelle Cason, Stephen Wall et al, Evaluation of Biotinylated PAMAM Dendrimer Toxicity in Models of the Blood Brain Barrier: A Biophysical and Cellular Approach, *Journal of Biomaterials and Nanobiotechnology*, 2011, 2, 485-493
39. Katare YK, Daya Ritesh P., Gray CS, Luckham Roger E, Bhandari J, Chauhan Abhay S, Mishra Ram K, Brain Targeting of a Water Insoluble Antipsychotic Drug Haloperidol via the Intranasal Route Using PAMAM Dendrimer, Mol. Pharmaceutics 2015, 12, 3380-3388
40. Dhanikula RS, Hildgen P. Influence of molecular architecture of polyether-co-polyester dendrimers on the encapsulation and release of methotrexate. Biomaterials 2007; 28:3140–52.
41. Dhanikula RS, Argaw A, Bouchard JF, Hildgen P. Methotrexate loaded polyether-copolyester dendrimers for the treatment of gliomas: enhanced efficacy and intratumoral transport capability. Mol Pharm 2008; 5:105–16.
42. Prieto MJ, Schilrreff P, Tesoriero MVD, Morilla MJ, Romero EL. Brain and muscle of Wistar rats are the main targets of intravenous dendrimeric sulfadiazine. Int J Pharm 2008; 360:204–12.
43. Singh S, Koland M, Formulation and evaluation of pulsatile drug delivery systems of glipizide for the management of type-II diabetes mellitus, Journal of Drug Delivery & Therapeutics. 2016; 6(1):11-18
44. Nyol S, Gupta MM, Immediate Drug Release Dosage Form: A Review, Journal of Drug Delivery & Therapeutics; 2013, 3(2), 155-161
45. Garg Ashish, Gupta M.M., Mouth Dissolving Tablets: A Review, Journal of Drug Delivery & Therapeutics; 2013; 3(2):207-214
46. Lin Y, Fujimori T, Kawaguchi N, et al. Polyamidoamine dendrimers as novel potential absorption enhancers for improving the small intestinal absorption of poorly absorbable drugs in rats. J Control Release 2011; 149:21–8.
47. Sadekar S, Ghandehari H. Transepithelial transport and toxicity of PAMAM dendrimers: implications for oral drug delivery. Adv Drug Deliv Rev 2012; 64:571–88.
48. Kolhe P, Misra E, Kannan RM, Kannan S, Lieh-Lai M. Drug complexation, in vitro release and cellular entry of dendrimers and hyperbranched polymers. Int J Pharm 2003; 259:143–60.
49. Thaxton CS, Georganopoulou DG, Mirkin CA. Gold nanoparticle probes for the detection of nucleic acid targets. Clin Chim Acta 2006; 363:120–6.
50. Dhakar RC, Maurya SD, Tilak VK, Gupta AK, A review on factors affecting the design of nasal drug delivery system, International Journal of Drug Delivery, 2011; 3 194-208
51. Dhakar RC, Nasal drug delivery: success through integrated device development, Journal of Drug Delivery & Therapeutics; 2011; 1(1):2-7.
52. Kapoor D, Vyas RB, Lad C, Patel M, Lal B, Site specific drug delivery through nasal route using bioadhesive polymers, Journal of Drug Delivery & Therapeutics. 2015; 5(1):1-9.
53. Dhuria SV, Hanson L R, Frey WH, II Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J. Pharm. Sci. 2010, 99:1654−1673.
54. Thorne RG, Frey WH, Delivery of neurotrophic factors to the central nervous system - Pharmacokinetic considerations. Clin. Pharmacokinet. 2001, 40, 907−946.
55. Capsoni S, Marinelli S, Ceci M, Vignone D, Amato G et al. Intranasal ″painless″ Human Nerve Growth Factors Slows Amyloid Neurodegeneration and Prevents Memory Deficits in App X PS1Mice. PLoS One 2012, 7, e37555.
56. Bahadur S, Pathak K. Physicochemical and physiological considerations for efficient nose-to-brain targeting. Expert Opin. Drug Delivery 2012, 9, 19−31.
57. Zhang QZ, Zha LS, Zhang Y. Jiang WM, Lu W, Shi ZQ, Jiang XG, Fu SK. The brain targeting efficiency following nasally applied MPEG-PLA nanoparticles in rats. J. Drug Targeting 2006, 14, 281−290.
58. Mistry A, Stolnik S, Illum L. Nanoparticles for direct nose-tobrain delivery of drugs. Int. J. Pharm. 2009, 379, 146−157.
59. Fazil M, Md S, Haque S, Kumar M, Baboota S, Sahni JK, Ali J. Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting. Eur. J. Pharm. Sci. 2012, 47, 6−15.
60. Piazza J, Hoare T, Molinaro L, Terpstra K, Bhandari J, Selvaganapathy PR, Gupta B, Mishra RK. Haloperidol-loaded intranasally administered lectin functionalized poly(ethylene glycol)- block-poly(D,L)-lactic-co-glycolic acid (PEG- PLGA)

nanoparticles for the treatment of schizophrenia. Eur. J. Pharm. Biopharm. 2014, 87, 30−39.
61. Kulhari H, Pooja D, Prajapati SK, Chauhan AS, Performance evaluation of PAMAM dendrimer based simvastatin formulations. Int. J. Pharm. 2011, 405, 203−209.
62. Perez AP, Mundina-Weilenmann C, Romero EL, Morilla MJ. Increased brain radioactivity by intranasal P-labeled siRNA dendriplexes within in situ-forming mucoadhesive gels. Int. J. Nanomed. 2012, 7, 1373−1385.
63. Kim, I.D.; Shin, J.H.; Kim, S.W.; Choi, S.; Ahn, J.; Han, P.L.; Park, J.S.; Lee, J.K. Intranasal delivery of HMGB1 siRNA confers target gene knockdown and robust neuroprotection in the postischemic brain. *Mol. Ther*. 2012, *20*, 829–839.
64. Perez, A.P.; Mundina-Weilenmann, C.; Romero, E.L.; Morilla, M.J. Increased brain radioactivity by intranasal P-labeled siRNA dendriplexes within *in situ*-forming mucoadhesive gels. *Int. J. Nanomedicine* 2012, *7*, 1373–1385.
65. Toub N, Malvy C, Fattal E, Couvreur P. Innovative nanotechnologies for the delivery of oligonucleotides and siRNA. Biomed Pharmacother 2006; 60:607–20.
66. de Martimprey H, Vauthier C, Malvy C, Couvreur P. Polymer nanocarriers for the delivery of small fragments of nucleic acids: oligonucleotides and siRNA. Eur J Pharm Biopharm 2009; 71:490–504.
67. Dufès C, Uchegbu IF, Schätzlein AG. Dendrimers in gene delivery. Adv Drug Deliv Rev 2005; 57:2177–202.
68. Galletti R, Masciarelli S, Conti C, Matusali G, Di Renzo L, Meschini S, et al. Inhibition of Epstein Barr Virus LMP1 gene expression in B lymphocytes by antisense oligonucleotides: uptake and efficacy of lipid-based and receptor-mediated delivery systems. Antiviral Res 2007; 74:102–10.
69. Tack F, Bakker A, Maes S, Dekeyser N, Bruining M, Elissen‐ Roman C, *et al*. Modified poly (propylene imine) dendrimers as effective transfection agents for catalytic DNA enzymes (DNAzymes). J Drug Target 2006; 14:69-86.
70. Pandita D, Santos JL, Rodrigues J, Pêgo AP, Granja PL, Tomás H. Gene delivery into mesenchymal stem cells: A biomimetic approach using RGD nanoclusters based on poly (amidoamine) dendrimers. Biomacromolecules 2011; 12:472-81.
71. Santos JL, Oliveira H, Pandita D, Rodrigues J, Pêgo AP, Granja PL, *et al*. Functionalization of poly (amidoamine) dendrimers with hydrophobic chains for improved gene delivery in mesenchymal stem cells. J Control Release 2010; 144:55-64.
72. Santos JL, Pandita D, Rodrigues J, Pêgo AP, Granja PL, Balian G, *et al*. Receptor‐ mediated gene delivery using PAMAM dendrimers conjugated with peptides recognized by mesenchymal stem cells. Mol Pharm 2010; 7:763-74.
73. Diaz-Mochon JJ, Fara MA, Sanchez-Martin RM, Bradley M. Peptoid dendrimers-microwave-assisted solid-phase synthesis and transfection agent evaluation. Tetrahedron Lett 2008; 49:923–6.
74. Hussain M, Shchepinov MS, Sohail M, Benter IF, Hollins AJ, Southern EM, et al. A novel anionic dendrimer for improved cellular delivery of antisense oligonucleotides. J Control Release 2004; 99:139– 55.
75. Vincent L, Varet J, Pille J-Y, Bompais H, Opolon P, Maksimenko A, et al. Efficacy of dendrimer-mediated angiostatin and TIMP-2 gene delivery on inhibition of tumor growth and angiogenesis: in vitro and in vivo studies. Int J Cancer 2003; 105:419–29.
76. Luo D, Haverstick K, Belcheva N, Han E, Saltzman WM. Poly(ethylene glycol)-Conjugated PAMAM dendrimer for biocompatible, high-efficiency DNA delivery. Macromolecules 2002; 35:3456–62.
77. Lee SC, Parthasarathy R, Botwin K, Kunneman D, Rowold E, Lange G, Klover J et al, Biochemical and immunological properties of cytokines conjugated to dendritic polymers. Biomed. Microdevices 2004. 6, 191–202.
78. Chaves F, Calvo JC, Carvajal C, Rivera Z, Ramirez Let al, Synthesis, isolation and characterization of Plasmodium falciparum antigenic tetrabranched peptide dendrimers obtained by thiazolidine linkages. J. Pept. Res. 2001. 58, 307–316.
79. Heegaard PMH, Boas U, an Sorensen NS, “Dendrimers for vaccine and immunostimulatory uses. A review,” *Bioconjugate Chemistry*, 2010; vol. 21, no. 3, pp. 405–418.
80. Crespo L, Sanclimens G, Pons M, Giralt E, Royo M, and Albericio F, “Peptide and amide bond-containing dendrimers,” *Chemical Reviews*, 2005; vol. 105, no. 5, pp. 1663–1681.
81. Tam JP, “Multiple antigen peptide system,” US5229490A, 1993.
82. Chaum E *et al*. Polyplex-mediated gene transfer into human retinal pigment epithelial cells in vitro. *J Cell Biochem* 1999; 76: 153–160.
83. Vandamme TF and Brobeck L, "Poly (amidoamine) Dendrimers as Ophthalmic Vehicles for Ocular Delivery of Pilocarpine Nitrate and Tropicamide," *J. Control. Rel*. 2005; 102 (1), 23–38.
84. Boas U, Heegaard PM, Dendrimers in drug research, Chemical Society Reviews, 2004; 33(1):43-63.
85. Shaunak S et. al., "Polyvalent Dendrimer Glucosamine Conjugates Prevent Scar Tissue Formation," *Nature Biotechnol.* 2004; 22 (8), 977–984.
86. Marano RJ et al., "Dendrimer Delivery of an Anti-VEGF Oligonucleotide into the Eye: A Long-Term Study into Inhibition of Laser-Induced CNV, Distribution, Uptake, and Toxicity,"*Gene Ther*. 2005; 12 (1), 1544–1550.
87. Yao WJ, Sun KX, Mu HJ et al., “Preparation and characterization of puerarindendrimer complexes as an ocular drug delivery system,” *Drug Development and Industrial Pharmacy*, vol. 36, no. 9, pp. 1027–1035, 2010.
88. Holden CA, Tyagi P, Thakur A et al., “Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs,” *Nanomedicine*, vol. 8, no. 5, pp. 776–783, 2012.
89. Thomas BJ, Finnin BC. 2004. The transdermal revolution. Drug Discov Today 9:697–703.
90. Wang ZX, Itoh Y, Hosaka Y, Kobayashi I, Nakano Y, Maeda I, Umeda F, Yamakawa J, Kawase M, Yag K. 2003. Novel transdermal drug delivery system with polyhydroxyalkanoate and starburst polyamidoamine dendrimer. J Biosci Bioeng 95: 541–543.
91. Cheng Y et al., "Transdermal Delivery of Nonsteroidal Anti-Inflammatory Drugs Mediated by Polyamidoamine (PAMAM) Dendrimers," *J. Pharm. Sci.* 2007; 96 (3), 595–602.
92. Chauhan AS et al., "Dendrimer-Mediated Transdermal Delivery: Enhanced Bioavailability of Indomethacin," *J. Control. Rel.* 2003, 90 (3), 335–343.
93. Kukowska-Latallo JF, Raczka E, Quintana A, Chen C, Rymaszewski M, Baker JR Jr. Intravascular and endobronchial DNA delivery to murine lung tissue using a novel, nonviral vector. *Hum Gene Ther*. 2000; 11:1385–1395.
94. Rudolph C, Lausier J, Naundorf S, Muller RH, Rosenecker J. In vivo gene delivery to the lung using polyethylenimine and fractured polyamidoamine dendrimers. *J Gene Med*. 2000; 2:269–278.
95. Bai S, Thomas C, Ahsan F. Dendrimers as a carrier for pulmonary delivery of enoxaparin, a low-molecular weight heparin. J Pharm Sci 2007; 96:2090–106.
96. Bai S, Ahsan F. Synthesis and evaluation of pegylated dendrimeric nanocarrier for pulmonary delivery of low molecular weight heparin. Pharm Res 2009; 26:539-48.
97. Shuhua B and Fakhrul A. Synthesis and Evaluation of Pegylated Dendrimeric Nanocarrier for Pulmonary Delivery of Low Molecular Weight Heparin. Pharmaceutical Research 2004; 26(3): 539-548.
98. Bai S, Gupta V, Ahsan F. Cationic liposomes as carriers for aerosolized formulations of an anionic drug: safety and efficacy study. Eur J Pharm Sci 2009; 38:165–71.
99. Chandel P, Raj Kumari, Kapoor A, Liquisolid technique: an approach for enhancement of solubility Journal of Drug Delivery & Therapeutics; 2013; 3(4):131-137
100. Jain NK, Gupta U, Application of dendrimer-drug complexation in the enhancement of drug solubility and bioavailability, Expert Opin Drug Metab Toxicol, 2008; 2003:1035-1045.

101. Mohammad N, Antony D, Crossing cellular barriers using dendrimer nanotechnologies, Current Opinion in Pharmacology, 2006; 6:522–527.

102. Hecht S, Fre´chet JMJ, Dendritic encapsulation of function: applying nature's site isolation principle from biomimetics to materials science, Angew. Chem., Int. Ed. Engl., 2001, 40:74–91.

103. Jiang DL, Aida T, A dendritic iron porphyrin as a novel haemoproteinmimic: effects of the dendrimer cage on dioxygenbinding activity, Chem. Commun, 1996, 1523–1524.

104. Sharma Deepak, Kumar Dinesh, Singh Gurmeet, Singh Mankaran, Rathore Mahendra Singh, A review on current advances in nanotechnology approaches for the effective delivery of anti-cancer drugs, Journal of Drug Delivery and Therapeutics, 2014; Special Issue1: "Drug Delivery using Nanomedicine & Nanotechnology" 67-71

105. Kapil A, Aggarwal G, Harikumar SL, Nanotechnology in novel drug delivery system, Journal of Drug Delivery & Therapeutics; 2014, 4(5), 21-28

106. Asadujjaman Md., Mishuk Ahmed Ullah, Novel approaches in lipid based drug delivery systems, Journal of Drug Delivery & Therapeutics; 2013; 3(4):124-130

107. Yadav G, Panchory H, Nanosponges: a boon to the targeted drug delivery system, Journal of Drug Delivery & Therapeutics. 2013; 3(4):151-155.

108. Lohumi A, Rawat S, Sarkar S, Sipai Altaf bhai, Yadav M. Vandana, A novel drug delivery system: niosomes review, Journal of Drug Delivery & Therapeutics. 2012; 2(5):129-135

109. Kesharwani P, Jain K, Jain NK. Dendrimer as nanocarrier for drug delivery. Progress in Polymer Science, 2014; 39(2):268-307.

110. Iyer AK, Khaled G, Fang J, Maeda H, Exploiting the enhanced permeability and retention effect for tumor targeting, Drug Discov. Today, 11 (2006) 812–818.

111. Myc A, Majoros IJ, Thomas TP, Baker JR Jr. Dendrimer‐based targeted delivery of an apoptotic sensor in cancer cells. Biomacromolecules 2007; 8:13-18.

112. Lai P-S, Lou P-J, Peng C-L, Pai C-L, Yen W-N, Huang M-Y, et al. Doxorubicin delivery by polyamidoamine dendrimer conjugation and photochemical internalization for cancer therapy. J Control Release 2007; 122:39-46.

113. Wiwattanapatapee R, Lomlim L, Saramunee K. Dendrimers conjugates for colonic delivery of 5-aminosalicylic acid. J Control Release 2003; 88:1-9.

114. Dodziuk H, Demchuk OM, Schilf W, Dolgonos G, Synthesis. NMR study of a first generation dendrimer having four branches involving four glycine and one carbomoyl-(3,7-dimethoxy-2- naphthalene) groups and attempts to complex it with α-, β- or γ-cyclodextrins. J Mol Struct 2004; 693:145–51.

115. Muhanna AMA, Ortiz-Salmerón E, GarcIa-Fuentes L, Giménez- MartInez JJ, Vargas-Berenguel A. Synthesis of peptide dendrimers based on a β-cyclodextrin core with guest binding ability. Tetrahedron Lett 2003; 44:6125–8.

116. Moghimi SM, Bonnemain B. 1999. Subcutaneous and intravenous delivery of diagnostic agents to the lymphatic system: Applications in lymphoscintigraphy and indirect lymphography. Adv Drug Deliv Rev, 37:295–312.

117. Gebbia V, Puozzo C. 2005. Oral versus intravenous vinorelbine: Clinical safety profile. Expert Opin Drug Saf, 4:915–928.

118. Duncan R, Izzo L. 2005. Dendrimer biocompatibility and toxicity. Adv Drug Deliv Rev 57:2215– 2237.

119. Dhakar Ram C, Maurya SD, Saluja V, From formulation variables to drug entrapment efficiency of microspheres: a technical review, Journal of Drug Delivery & Therapeutics; 2012, 2(6), 128-133.

120. Kukowska-Latallo JF, Candido KA, Cao ZY, Nigavekar SS, Majoros IJ, Thomas TP, Balogh LP, Khan MK, Baker JR. 2005. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 65:5317–5324.

121. Bhadra D, Bhadra S, Jain S, Jain NK. 2003. A PEGylated dendritic nanoparticulate carrier of fluorouracil. Int J Pharm 257:111–124.

122. Chauhan AS, Jain NK, Diwan PV, Khopade AJ. 2004. Solubility enhancement of indomethacin with poly(amidoamine) dendrimers and targeting to inflammatory regions of arthritic rats. J Drug Target 12:575-583.

123. Sanghai B., Aggarwal G., & HariKumar S. Solid self microemulsifying drug deliviry system: a review. Journal of Drug Delivery and Therapeutics, 2013; 3(3), 168-174.

 ISSN: 2250-1177 CODEN (USA): JDDTAO