Dendrimer as a carrier for ocular drug delivery

Tariq Baig*, Hammad Sheikh, Ankur Srivastava, Pushpendra K. Tripathi

Rameshwaram Institute of Technology & Management, Lucknow, Uttar Pradesh, India

Received 20 July 2014; Accepted 30 July 2014

INTRODUCTION:
Dendrimers are novel synthetic polymeric systems having enhanced physical and chemical properties due to their unique 3-D structural design. Dendrimers are well defined size, shape, molecular weight and monodispersity [1]. Dendrimer chemistry was first introduced in 1978 by Fritz Vögtle and co-workers. He synthesizes the first “cascade molecules”. In 1985, Donald A. Tomalia synthesizes the first family of dendrimers. The word "dendrimer" originate from two words. Firstly, the Greek word dendron, meaning tree, and secondary meros, mean part. At the similar time, Newkome’s group independently report synthesis of parallel macromolecules. They called them “arborols” from the Latin word ‘arbor’ also meaning a tree. The term “cascade molecule” is also used, but ‘dendrimer’ is the best established one. Due to their multivalent and mono disperse nature; dendrimers have enthused wide interest in the field of chemistry and biology, particularly in application like drug delivery, gene therapy and chemotherapy [2]. Dendrimers are precisely defined, synthetic material that is approximately 2-10 nanometres in diameter. They are hyper branched and monodisperse three-dimensional molecules with defined molecular weights, large numbers of functional groups on the surface and well-established host-guest entrapment properties. They are made up of layers of polymer surrounding a central core.

ABSTRACT
The foremost challenges face by today’s researcher is ocular drug delivery. Delivery of drug to the targeted ocular tissue is restricted by various ocular barriers. To conquer the ocular drug delivery barriers and get better ocular bioavailability, different conventional and novel drug delivery agents have been used. Dendrimer act as a drug delivery agent for promising, safe and selective drug delivery option. It’s extremely high selective nature for target the desired amount of drug to the tissue is most essential property and holds a promising future for the treatment of various ophthalmic diseases. Dendrimer shows physiochemical properties like pH, osmolality, and viscosity etc that are more compatible with ocular dosage form to formulate different formulations for ocular diseases. The important advantages of dendrimer in ocular drug delivery are persistence in corneal residence time, which can improve the bioavailability of drug, and introduce in the form of eye drops. Dendrimers help in achieving increased bioavailability, sustained, controlled as well as targeted release of drug.

Keywords: dendrimer, bioavailability, ocular drug delivery, hydrogels.
Advantages of ocular drug delivery systems: Emerged. These approaches are essential because the anterior and posterior segments of the eye comprise recently new drug candidates, including biologics, for the growth of new technologies in ocular drug delivery and very common, especially in the aging population. A rapid-related macular degeneration (AMD), and glaucoma, are uncommon, whereas others, such as cataracts, age-diseases, lots of which lead to visual impairment and millions of people bear from a wide range of ocular ailments which affected to eye and one can defect the eye sight also. Consequently many ophthalmic drug delivery systems are available. These are classifying as conventional and non-conventional (newer) drug delivery systems. Generally available ophthalmic preparations are eye drops and ointments about 70% of the eye dosage formulations in market. But these preparations after instilled into the culde-sac are quickly drained away from the ocular cavity due to tear flow and lachrymal nasal drainage. [8-10] Only a diminutive amount is available for its therapeutic effect resulting in frequent dosing. So conquer to these problems newer pharmaceutical ophthalmic formulation such as in-situ gel, nanoparticle, liposome, nanosuspension, microemulsion, intophoresis and ocular inserts have been developed in last three decades enhance the bioavailability of the drug as a sustained and controlled manner [11-13].

Millions of people bear from a wide range of ocular diseases, lots of which lead to visual impairment and ocular blindness. Certain ocular diseases are quite uncommon, whereas others, such as cataracts, age-related macular degeneration (AMD), and glaucoma, are very common, especially in the aging population. A rapid growth of new technologies in ocular drug delivery and new drug candidates, including biologics, for the treatment of these challenging diseases in the anterior and posterior segments of the eye comprise recently emerged. These approaches are essential because the eye has many unique barriers to drug delivery [14].

Advantages of ocular drug delivery systems: Ophthalmic drug delivery system has many advantages as given below [15-20].
1. Exact dosing: to diminish the adverse effects of pulse dosing which are produce by conservative systems.
2. To fabricate sustained and controlled release of drug.
3. Boost the corneal contact time to get better the ocular bioavailability of drug. This could be achieving through effective adherence to corneal surface.
4. Help to board into the ocular globe, to guard the other ocular tissues.
5. To break out the protective barriers like drainage, lacrimation and conjunctival absorption.
6. Drug relevance has needle free and administered devoid of any requirement of trained personal assistance for the application i.e. self medication thus improve the patient compliance as patient compliances as compared to parenteral routes.
7. Good hydrophilic diffusion and low molecular weight drugs can be experiential in the eye.
8. Quick absorption and fast onset of action due to large surface area and high vascularisation. Administration of ocular drug would be valuable in emergency therapy as a substitute to other routes.
9. To keep away from the hepatic first pass metabolism and thus dose decrease has potentiated as compared to oral delivery.

Disadvantages of ocular drug delivery systems: Disadvantages of ocular drug delivery systems: Ocular drug delivery has some limitation as review below [21-23].
1. The physiological constraint has incomplete permeability of cornea resultant into low absorption of ophthalmic drugs.
2. A major segment of the administered dose drains into the lacrimal duct and thus can grounds surplus systemic side effects.
3. The quick elimination of the drug through the eye blinking and tear flow results in a short period of the therapeutic effect that results in a frequent dosing regimen.

Use of dendrimer in ocular drug Delivery: Dendrimers has been used to observe their role in release of therapeutics and imaging agents [104]. Dendrimers can recover drug’s water solubility, bioavailability, and biocompatibility and can be used to improve drug permeability [24, 25]. In Ocular dendrimetic formulations have developed to lack cytotoxicity or irritation [26]. For ocular drug delivery, formulator must be sure about the protection of dendrimers because severe side effects may arise due to cytotoxicity at the ocular tissues. In order to conquer the potential toxicity of the dendrimers, ophthalmologists have contributed in the scientific progression together with the chemists, formulation scientists and engineers [27, 28]. Dendrimers are valid for several purposes such as drug delivery, gene delivery, peptide delivery, antioxidant delivery, biomedical imaging, and genetic testing in ophthalmology [29]. It also enables to convey into and out of the cells. PAMAM dendrimers can have dissimilar cell entry pathways, which depend on the functional groups on the surface. Anionic PAMAM dendrimers are endocytosed mostly by the caveolin-mediated process, while neutral and cationic dendrimers are internalized in cells subsequent to a clathrin-mediated process. These pathways can be essential in passage of the epithelial and retinal barrier in the cornea and retina [30, 31].

Dendrimeric poly guanidilyated translocators (DPTs), which go to tritolyl branches and surface guanidine groups has been used as haulier for ophthalmic drug delivery of gatifloxacin, which is a “fourth generation
fluoroquinolone” permitted for the treatment of conjunctivitis. The result shows that the DPT formed stable gatifloxacin complexes and enhance solubility, permeability, anti-MRSA activity [32].

Intraocular tumours such as retinoblastoma symbolize complications with high metastatic potential. In this study, PAMAM dendrimers with carboxyl end groups (G3.5-COOH) have been used for drug liberation to intraocular tumours for extensive half life and sustained delivery of carboplatin with lower therapeutic toxicity. Carboplatin-loaded PAMAM dendrimer complex were exposed in a transgenic murine retinoblastoma model, by subconjunctival administration. It was observed that the carboplatin loaded dendrimer nanoparticles has cross the sclera and retained for an absolute period of time in the tumour vasculature, which provide a sustained treatment effect [33].

PAMAM dendrimers used as ophthalmic vehicles for delivery of pilocarpine nitrate and tropicamide, for miotic and mydriatic action. In this study, mean ocular residence time for fluorescein in saline and in PAMAM solutions is studied in rabbit eye. Fluorescein in 0.2% w/v Carbopol solution was used as reference bioadhesive polymer. The mean ocular residence time was considerably superior in case of PAMAM solutions and 0.2% w/v Carbopol solution compare to saline. Therefore, the use of dendrimers might be another option for increasing ocular residence time and therapy enhancing ocular bioavailability and achieve better therapeutic outcomes. For instance, PAMAM dendrimers when co-administrated with pilocarpine nitrate and tropicamide, show elevated miotic and mydriatic activity in albino rabbits [34].

In order to evade scar tissue arrangement after glaucoma filtration surgery, conjugates of modified PAMAM dendrimers with glucosamine (DG) and glucosamine 6-sulfate (DGS) were synthesize to apply immune-modulatory and anti-angiogenic activities, respectively. The subconjunctival administration of these customized conjugates in rabbit model of glaucoma filtration surgery have exposed significant inhibition of pro-inflammatory and pro-angiogenic responses and as a result to reduced scar tissue formation. The results obtained from the experiment indicate that the ocular administration of DG and DGS might be efficient and safe in clinical practice in avoiding scar tissue formation post glaucoma filtration surgery [30].

Topical administration of puerarin PAMAM dendrimer complex:
For the study of pharmacokinetic properties of puerarin poly(amide amine) (PAMAM) dendrimer complex, a sensitive liquid chromatography mass spectrometry method (LC-MS/MS) was developed and validate to conclude puerarin in rabbit aqueous humour with microdialysis sampling. Astilbin was used as the internal standard. The linear array for puerarin was from upto 2 to 1000ng/mL (r=0.9986) base on 20μL of aqueous humour. The coefficients of variations for intra-day and inter-day precisions are less than 10.0%, and the relative error of accuracy was within ±6.3%. The average extraction recovery of puerarin varied from 80.4% to 85.5%. Microdialysis provides an absolute concentration vs. time profile. An important difference was observed in main pharmacokinetic parameters of Cmax, AUC and t1/2 involving puerarin solution and puerarin PAMAM dendrimer complex. Complex formation resulted in a noticeable increase in bioavailability of puerarin after topical administration to rabbit according to the LC-MS/MS assay method. [35]

Biocompatible dendrimer used in the treatment of retinal degeneration:
Biocompatible dendrimers are well-defined nanosizes particles which are gradually used as carriers for drug delivery. 5-Aminosalicylic acid (5-ASA) is an FDA-approved therapeutic agent which is newly found to be effective in treating retinal degeneration of animal models. In this study, a water-soluble dendrimer conjugate of 5-ASA (AGFB-ASA) was intended to treat such retinal degeneration. The drug was conjugated with generation 2 (G2) lysine dendrimer with a silsesquioxane core (nanoglobule) by using a hydrolyzable Schiff base spacer. Incubation of nanoglobular G2 dendrimer conjugates contains a 4-formylbenzoate (FB) Schiff base spacer in pH 7.4 phosphate buffers at 37 °C slowly released 5-ASA. Drug liberate from the dendrimer conjugate was considerably slower from the low molecular weight free Schiff base of 5-ASA (FB-ASA). 5-ASA discharge from the dendrimer conjugate was dependent relative on stearic hindrance around the spacer. After intra-peritoneal injection, the nanoglobular 5-ASA conjugate provide more effective on 7th day protection against light-induced retinal deterioration at a reduced dose than free 5-ASA in Abca4 (-/-) Rdh8 (-/-) mice. The dendrimer 5-ASA conjugate with a degradable spacer might be a good applicant for controlled delivery of 5-ASA to the eye for treatment of retinal degeneration. [36]

Hydrogel/PLGA nanoparticle platform for sustains drug delivery to measure antiglaucoma effects for topical administration:
In this study, a novel hybrid polyamidoamine (PAMAM) dendrimer hydrogel/poly(lactic-co-glycolic acid) (PLGA) nanoparticle platform (HDNP) for codelivery of two antiglaucoma drugs, brimonidine and timolol maleate has
been performed and this platform was not cytotoxic to human corneal epithelial cells. Cellular uptake of Nile red-encapsulating PLGA nanoparticles was extensively increased by dendrimer hydrogel. A long-lasting residence time of nanoparticles was established through examination of FluoSpheres loaded into dendrimer hydrogel. Both brimonidine and timolol maleate be slowly released in-vitro over a period of 28-35 days. Topical administration of one eye drop (30 μL of 0.7% w/v brimonidine and 3.5% w/v timolol maleate) in normotensive adult Dutch-belted male rabbits, the HDNP formulation resulting in a sustained and effective IOP decrease (18% or higher) for 4 days. Moreover, the HDNP formulation in aqueous humour, cornea, and conjunctiva up to 7 days as compared to saline, DH, and PLGA nanoparticle dosage forms, without inducing ocular inflammation or distress. This new proposal is able for enhancing drug bioavailability and sustaining efficient IOP reduction over an extended period of time. This newly developed platform can deeply decrease dosing frequency for topical formulations, and thus improved long-term patient compliance and reducing economic costs. [37]

**Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs:**

Dendrimer hydrogel (DH), prepared from ultraviolet-cured polyamidoamine dendrimer G3.0 with polyethylene glycol (PEG, 12,000 Da)-acrylate chains (8.1% w/v) in pH 7.4 phosphate buffered saline (PBS) for the delivery of brimonidine (0.1% w/v) and timolol maleate (0.5% w/v), two antiglaucoma drugs. DH was found to be mucoadhesive to mucin particles and is nontoxic to human corneal epithelial cells. DH improved the PBS solubility of brimonidine by 77.6% and constant the in vitro release of both drugs over 56-72 hours. As compared to eye drop formulations (PBS-drug solutions), DH brings about extensively higher human corneal epithelial cells uptake and significantly increased bovine corneal transport for both drugs. DH increased timolol maleate uptake in bovine corneal epithelium, stroma, and endothelium by 4.4- to 4.6-fold. DH can improve the delivery of antiglaucoma drugs in multiple aspects and represent a novel platform for ocular drug delivery. Dendrimer hydrogel was studied as agent for simultaneous delivery of two anti-glaucouma drugs, one hydrophobic and other hydrophilic. Advantage over standard PBS-based formulation was clearly established for both drugs. [38]

**Dendrimer crosslinked collagen as a corneal tissue engineering scaffold:**

Generation 2 polypropyleneimine octaamine dendrimers were used to generate highly crosslinked collagen with mechanical properties that would make it appropriate for use as a corneal tissue-engineering scaffold. Crosslinking of a highly concentrated collagen solution (2-4%) was effected using the water-soluble carbodiimide 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). The multifunctional dendrimers were introduced as novel multifunctional crosslinkers after the activation of the carboxylic acid groups of glutamic and aspartic acid residues in collagen. Glutaraldehyde, a common collagen crosslinker, was used as comparison, as was EDC, itself an alternative crosslinker, which forms "zero-length or self-crosslinking". The mechanical properties resultant gels were determined. Young's modulus of the dendrimer crosslinked gels was significantly higher than that observed with the other crosslinkers, increasing to 5 MPa compared with 0.1 MPa for the EDC crosslinked gels. Transmission electron microscopy (TEM) analysis of the gels demonstrated the presence of fibrils in the thermally gelled collagen controls; no fibrils were observed in the dendrimer crosslinked gels. As a result, the optical transparency of the dendrimer crosslinked collagen was significantly better than that of the collagen thermal gels. The EDC and glutaraldehyde crosslinked gels were generally less transparent than those crosslinked with the dendrimers. Glucose permeation results demonstrated that the dendrimer crosslinked collagen had higher glucose permeability than natural human cornea. Dendrimer crosslinked collagen gels supported human corneal epithelial cell growth and adhesion, with no cell toxicity. In comparison, some potentially cytotoxic effects were observed with glutaraldehyde crosslinked collagen. Overall, the dendrimer crosslinked collagen gels showed promising properties that suggest that these might be suitable scaffolds for corneal tissue engineering and potentially other tissue engineering applications. [39]

**Designing hydrogel adhesives for corneal wound repair:**

Today, corneal wounds are repaired using nylon sutures. Yet there is a number of complications associated with suturing the cornea, and thus there is interest in an adhesive to replace or supplement sutures in the repair of corneal wounds. We are designing and evaluating corneal adhesives prepared from dendrimers—single molecular weight and highly branched polymers. We have explored two strategies to form these ocular adhesives. The first involves a photocrosslinking reaction and the second uses a peptide ligation reaction to couple the individual dendrimers together to form the adhesive.
These adhesives were successfully used to repair corneal perforations, close the flap produced in a LASIK procedure, and secure a corneal transplant. [40]

**Improved the biological stability of collagen with incorporation of PAMAM dendrimer:**
The crosslinking methods of collagen using glutaraldehyde (GTA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) are frequently performed in biomedical applications, but both methods still have their own disadvantages, including the GTA cytotoxicity and low degree of EDC/NHS crosslinking. In this study, we incorporated polyamidoamine (PAMAM) dendrimer with surface amine groups into the two aforementioned crosslinking methods to improve the biostability and structural integrity of collagen. Fifty micromolar of dendrimer concentration was found to have negligible in vitro cytotoxicity and was used for EDC and GTA crosslinking of collagen. The collagenase digestion assay showed that the collagen scaffolds crosslinked in the presence of PAMAM exhibited a higher denature temperature and higher resistance against collagenase digestion compared with their counterparts without dendrimer. Cell proliferation with human conjunctival fibroblasts showed that the incorporation of PAMAM in EDC crosslinking significantly increased the proliferation. All the crosslinked scaffolds also exhibited higher structural stability than the noncrosslinked scaffold. Crosslinking with EDC and PAMAM together yielded substantially higher proliferation and may be a suitable collagen scaffold for biomedical applications. [41]

**Designing dendrimers for ocular drug delivery:**
New series of phosphorus-containing dendrimers, having one quaternary ammonium salt as core and carboxylic acid terminal groups have been synthesized from generation 0 (3 carboxylic acid terminal groups) to generation 2 (12 carboxylic acid terminal groups). These dendrimers react with the neutral form of carteolol (an ocular anti-hypertensive drug used to treat glaucoma) to afford ion pair (saline) species. The solubility in water of these charged dendrimers depends on the generation considered: generation 0 (3 carteolol) is well soluble, whereas generation 1 (6 carteolol) and generation 2 (12 carteolol) are poorly soluble. These dendrimers have been tested in vivo, as vehicle for ocular drug delivery of carteolol to rabbits. [42]

**Effect of poly (amidoamine) dendrimers on corneal penetration of puerarin:**
The aim of this study was to investigate the effect of Poly (amidoamine) (PAMAM) dendrimers on corneal permeation of puerarin (PUE). Permeation studies were performed using excised cornea of rabbits by a Valia-Chien diffusion apparatus. Drug-treatment studies were carried out by measuring the penetration of puerarin on PAMAM-PUE complex, and cornea-treatment studies were carried out by measuring the penetration of puerarin on PAMAM dendrimer pretreated cornea in puerarin solution. The results showed that the permeability coefficient of puerarin in PAMAM-PUE physical mixture was enhanced by 2.48 (G3), 1.99 (G4) and 1.36 (G5) times on average, respectively compared to control. However, no significant permeability enhancement of puerarin in PAMAM-PUE complex was found compared to control. This may attribute to free drug concentration was lower in PAMAM-PUE complex which served as a depot and exhibited slow-released behavior of drug. Cornea-treatment studies showed that the lag time of puerarin was decreased, while the cumulative amount within 2.5 h (Q(2.5)) and the permeability coefficient of puerarin increased compared to control. The permeability coefficient of puerarin was linear correlated to the molecular weight of PAMAM dendrimer ($r^2=0.99$). This indicates that higher generation of PAMAM dendrimer more easily interact with cornea or loosen the epithelium cell junctions than lower generation to increase the flux of puerarin. Overall, the study showed that PAMAM dendrimer increased the corneal permeation of puerarin mainly by altering the corneal barrier. [43]
Table 1: Applications of dendrimers for ocular drug delivery:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Treatment</th>
<th>Dendrimer type</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piocarpine nitrate and tropicamide (topical)</td>
<td>Myosis and mydriasis</td>
<td>PAMAM G1.5-4</td>
<td>Increased corneal residence and reduced intraocular pressure [44]</td>
</tr>
<tr>
<td>Carteolol (topical)</td>
<td>Glaucoma</td>
<td>Phosphorus containing dendrimers</td>
<td>Increased corneal residence and decreased toxicity and intraocular pressure [45]</td>
</tr>
<tr>
<td>Concanavalin A (Topical Photodynamic therapy)</td>
<td>Intraocular tumors and retinoblastoma</td>
<td>Porphyrin glycodendrimers</td>
<td>Enhanced targeting and reduce toxicity [30]</td>
</tr>
<tr>
<td>Puerarin</td>
<td>Ocular hypertension and cataract</td>
<td>PAMAM</td>
<td>Increased bioavailability [46]</td>
</tr>
<tr>
<td>Fluocinolone acetonide (Intravitreal injection)</td>
<td>Retinal neuro-inflammation</td>
<td>PAMAM G4-OH</td>
<td>Reduced inflammation [47]</td>
</tr>
</tbody>
</table>

CONCLUSION:
Various approaches have been investigated in ophthalmic delivery system in the earlier period. Several new approaches in advanced ocular delivery system such as micro needle, implants, ultrasound technology, and nano technology including dendrimers, nanoparticle as well as gene transfer technique. But dendrimers can work as a useful tool for ocular drug delivery to enhance the persistence in corneal residence time which improves the bioavailability of drugs to overcome the ophthalmic problems. Also the problem of biocompatibility and toxicity can be overcome by careful surface engineering. This novel and advanced technology in ocular drug delivery provide protection and effective mean of therapy.

ACKNOWLEDGEMENT:
The authors wish to thank Dr. Pushpendra K. Tripathi (Director), Department of Pharmacy, Rameshwaram Institute Of Technology & Management, Lucknow, for their kind support and encouragement to carry out this work.

REFERENCES:


