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Impacts of Nanomedicines in Ocular Pharmacotherapy

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ABSTRACT

Introduction: The integrity of the cells/tissues in anterior and/or posterior segments of the eye plays a crucial role in biofunctions of the vision. To maintain ocular homeostasis, selective restrictiveness of the ophthalmic membranes and barriers control must act on shuttling of biomolecules. Thus, not all attempts to apply de novo nanotechnology approaches for ocular pharmacotherapy have met with the same successes as those cited here in this review, and sometimes these novel technologies tools provoke a great deal of challenges and hurdles mainly because of functional presence of these barriers. ***Methods:*** Recent published articles related to applications of ocular nanomedicines were reviewed and highlighted in this review article. ***Results:*** It seems the emergence of nanomedicines have arisen great hopes for ophthalmic pharmacotherapy, in which nanostructured medicines are expected to be able to cross the restrictive barriers of the eye. Although such fast inauguration of ocular nanomedicines will literally convey new challenges in the regulatory and translational processes, it will also grant a prolific platform from which many exciting, and yet unimagined, applications of biomedical nanotechnology will emerge for pharmacotherapy of the eye. ***Conclusion:*** This review provides recent advancements on ocular nanomedicines.

Introduction

The unique structural and functional properties of the eye are synchronized by visual cells and transparent tissues. The regulatory mechanism of this organ relies mainly on tight cellular barrier between eye's anterior and posterior segments which controls fluids and solutes traverse through membranes (the schematic illustration of the eye structure and main ocular barriers are demonstrated in Fig. 1.). Similarly, drug transport via these barriers is also highly controlled and limited; hence, application of novel drug delivery/ targeting strategies for effective pharmacotherapy seems to be crucial.

Recent advances in gene based therapeutics and novel nano-sized delivery targeting/delivery agents are generating new insights for the ocular disease therapy. However, efficient delivery and adequate bioavailability of such medications should be verified (Barar *et al.* 2008; Urtti 2006).

Blood ocular barriers

Normal ocular structure and visual function properties are maintained by blood ocular barriers consisting of two main components (i.e. blood aqueous barrier (BAB) and blood retinal barrier (BRB)). This barrier physically separates blood vessels from internal segment of eye, controlling passage of any particle/chemical into ocular tissues. As illustrated in Fig. 1, ocular medications administered via local or systemic routes must overcome this barrier to achieve adequate concentration and maintenance in retina and vitreous. Furthermore, blood ocular barrier maintains tissue/fluid composition and produces aqueous humor (Cunha-Vaz 1997a). The control of inflow/outflow of aqueous humor provides the sufficient pressure inside the eye (Fischbarg 2006). In the following sections, the location of blood aqueous and blood retinal barriers will be discussed and their functionality to impede drug transport will further be reviewed.

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Blood-aqueous barrier

The BAB is formed by tight junctions of iris vascular endothelium and non-pigmented layer of ciliary epithelial cells (Fig. 1). This structure is located in the anterior part of the eye, preventing undesired traverse of exogenous materials into the ocular posterior segment and providing transparency and chemical equilibrium of the ocular fluids (Cunha-Vaz 1997b; Freddo 2001).It is of note that iris blood vessels withstand macromolecule (e.g. horse radish peroxidase with a molecular size of 40 kDa) passage, whilst capillary of ciliary body is less restrictive and allows favored outward traverse of substances toward systemic circulation. Moreover passive transport through BAB, results in rapid elimination of substances with small molecular size and more lipophilicity as compared to large and hydrophilic molecules. For example, the clearance of pilocarpine is13.0 µl/ml in rabbits whereas inulin clearance is close to the rate of aqueous humor turnover (i.e. 3.0–4.7 ml/min) (Conrad and Robinson 1977).

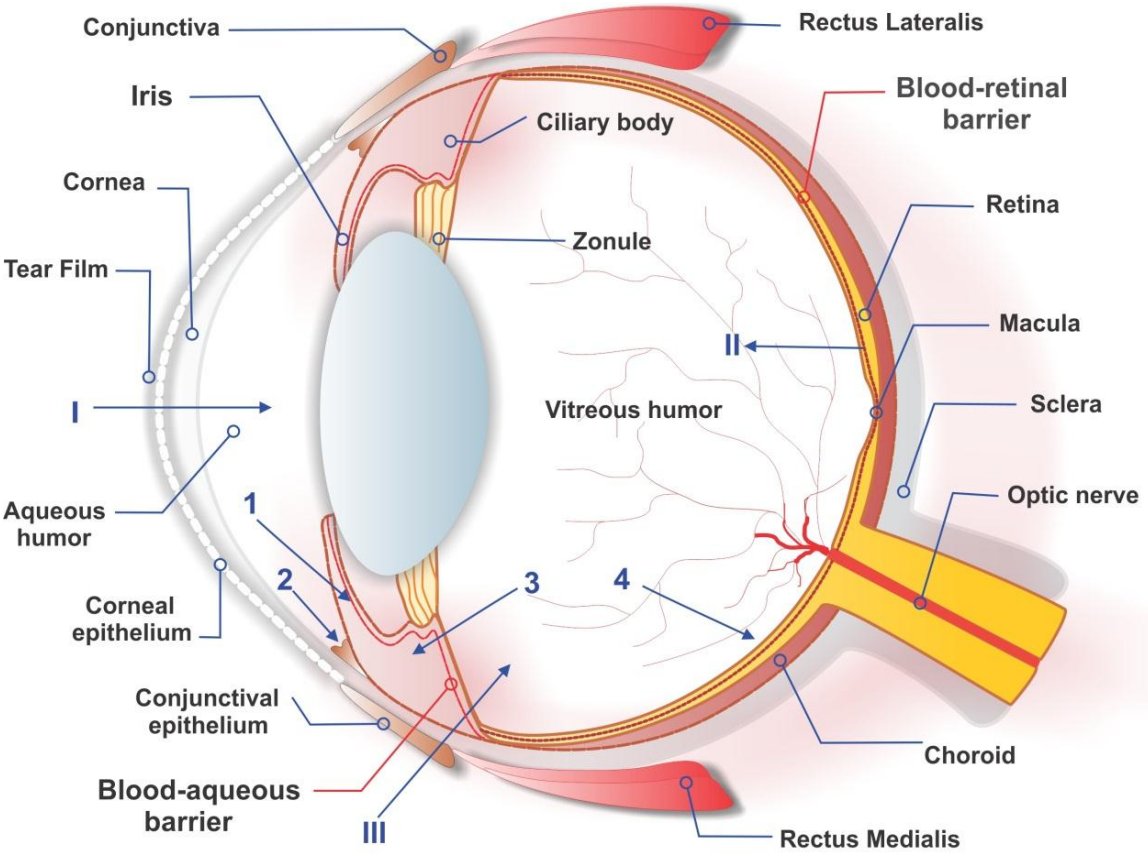

Fig. 1. Schematic illustration of the main structure of the eye and the ocular barriers. The primary physiologic blockage against installed drugs is the tear film. Cornea is the main route for drug transport to the anterior chamber (I). The retinal pigment epithelium and the retinal capillary endothelium are the main barriers for systemically administered drugs (II). Intravitreal injection is an invasive strategy to reach the vitreous (III). The administered drugs can be carried from the anterior chamber away either by venous blood flow after diffusing across the iris surface (1) or by the aqueous humor outflow (2). Drugs can be removed from the vitreous away through diffusion into the anterior chamber (3), or by the blood–retinal barrier (4). The image was adopted with permission from (Barar *et al.* 2009).

The blood-retinal barrier

The blood-retinal barrier (BRB), which locates in the posterior part of the eye, contains two types of cells, i.e. 1) the retinal capillary endothelial (RCE) cells, and 2) the retinal pigment epithelial (RPE) cells. These cells form the inner and outer BRB, respectively. Basically, the specialized transport processes and the restrictive barrier functions RPE selectively control the transportation of nutrients/compounds, by which functionalities only designated nutrients can be traversed between choroid and retina (Duvvuri *et al.* 2003; Mitra *et al.* 2006). The inner BRB covers the lumen of retinal

capillaries that are able to selectively protect the retina from circulating molecules of the blood. In fact, the RCE cells possess intercellular tight junctions which are formed upon the intercellular communications of the RCE cells with the glial cells (Gardner *et al.* 1999), similar to that in the blood–brain barrier (BBB) by brain microvasculature endothelia (Janzer and Raff 1987). Due to the functional expression of tight junctions and intercommunications with astrocytes and Müller cells, RCE cells display biological characteristics similar to BBB (the brain capillary endothelial cells associated with pericytes and astrocytes) with trans-endothelial electrical resistance (TEER) value of 1500-2000 ($\Omega.cm^2$) (Barar and Omidi 2008; Omidi *et al.* 2003a; Omidi *et al.* 2008; Smith *et al.* 2007).

Given these facts, it appears that the satisfactorily delivery and efficient pharmacological effects of drugs within the vitreous and the retina require systemic or intravitreal drug administration. Nevertheless, systemic application via oral or intravenous administration necessitates very high doses of the drug because the blood flow and restrictive functionality of BRB allow only very trivial fraction of the drug to reach the posterior chamber (typically only 1-2% of the concentration in the plasma). But, it should be evoked that administration of large portion of the drug can be associated with inadvertent adverse consequences (Selvin 1983).

In short, damage of the normal BRB appears to be the common feature to many retinal degenerative diseases such as diabetes. Thus development of novel modalities to prevent loss of barrier properties or restore barrier properties is considered as a high priority in ophthalmology.

Strategies to overcome blood ocular barriers

In recent years, for treatment of many ocular disorders, there has been a profound shift towards implementation of more efficient treatment paradigms. For example, the neurodegenerative disorder "glaucoma" which is associated with elevated intraocular pressure has affected many patients' lives, while its treatment has fortunately moved from the management of intraocular pressure to the prevention of neurodegeneration and maintenance of retinal function. The artificial tears is no longer the main treatment for the dry eye which is a common cause of patient visits to eye care specialists damaging the ocular surface. It is now being controlled with Restasis® (cyclosporin A ophthalmic emulsion), which targeted the immune component of the disease (Attar *et al.* 2005).

In ocular pharmacotherapy, the biggest challenge is achievement of the preferred concentration at the intended ocular tissue. To tackle this issue, a variety of conventional ocular drug delivery systems have been developed for the production of effective ophthalmic drug formulations. Most of these ophthalmic drugs are delivered to the eye via aqueous vehicles. Nonetheless, the aqueous vehicles exhibit poor ocular bioavailability due to rapid drainage, lacrimation and tear turnover, and if penetration occurs, only a short duration of action will be observed (Hillaireau *et al.* 2006; Lang 1995).

Moreover, application of many potentially active ophthalmic compounds is seriously limited because of their very low water solubility. They, accordingly, need to be administered either through alternative routes or by optimized delivery system. Among various approaches for improving ophthalmic delivery of lipophilic drugs, hydrogels, microparticles, nanoparticles and liposomal formulations have been shown to favor topical targeting and to improve drug bioavailability (Patravale *et al.* 2004). Of these DDS, nanoformulations have raised promising potential for efficient ocular delivery (Bucolo *et al.* 2004). In fact, the colloidal nanoparticle drug carriers emerge to be useful for ocular absorption enhancement through various mechanisms including: prolonged drug residence time in the cornea and conjunctival, sustained drug release from the delivery system, and reduced pre corneal drug loss (Bu *et al.* 2007). Surprisingly, over the past two decades, nanoformulations of ophthalmic drugs have not yet been undertaken in clinical practice as fast as it was expected to be.

Drug-polymer nanoformulations

Drug delivery systems with biodegradable/bioerodible polymers can provide a significant advantage over the non-degradable systems because the entirety is eventually absorbed by the body, eliminating the need for subsequent removal. However, these polymers are predisposed and time dependent due to erosion, which can occur through the following mechanisms: a) cleavage of the cross-linked or water-soluble backbone in the cross-linked water-soluble macromolecules, b) hydrolysis, ionization, or protonation of pendant groups in the water-insoluble macromolecules, and c) hydrolytic cleavage of labile bonds in the polymer backbone high-molecular-weight, water-insoluble macromolecules (Kimura and Ogura 2001).

The pattern of drug release largely depends upon the association of drug with polymers since two approaches can be undertaken for formulation, i.e. a drug core surrounded by a rate-controlling biodegradable membrane, or the drug dispersed within polymer(s). Of the polymer based systems, the nanoparticles are colloidal drug carrier systems with a size range of 10 to 1000 nm, while nanospheres are solid matricial structures carrying drug molecules within the matrices and/or adsorbed on the surfaces of the colloidal carriers and finally nanocapsules are small capsules with a

central core surrounded by a polymeric shell with dissolved/adsorbed drug molecules in core/surface interface.

Upon our literature survey, the most commonly used polymers in the ophthalmic drug formulations are: poly(alkyl cyanoacrylates), PCL, and poly(lactic acid)/poly(lactic-co-glycolic acid) (PLA, PGA, PLGA). Moreover, some others such as chitosan (CS), ERL/ERS, PS, and poly(acrylic acid) (PAA) as well as the bovine serum albumin (BSA) has also been exploited for ocular delivery as drug-loaded nanoformulations. Fig. 2 represents the chemical structures of some important polymers used in the preparation of nanoformulations.

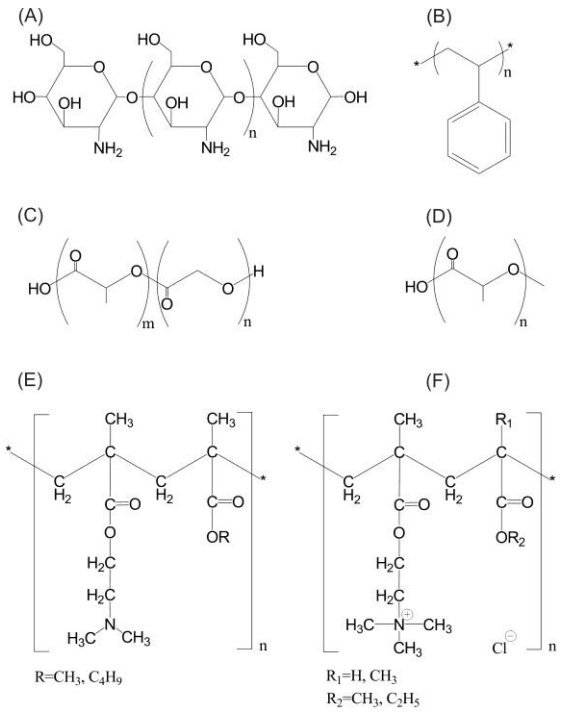

Fig. 2. Chemical structures of selected polymers used for preparation of ocular nanomedicines. A) Chitosan. B) Polystyrene. C) *PLGA* (copoly lactic acid/glycolic acid). D) PLA(poly lactic acid). E) Eudragit E. F) Eudragit RL/RS .

Given that the surface of the ocular tissues (e.g., cornea and conjunctiva) is negatively charged, the cationic colloidal nanoparticles are expected to confer better penetration potential through the ocular membranes and barriers. Of the polymers used for ocular delivery, few polymers (CS, ERL and ERS) grant positively charged nanoparticles (Bu *et al.* 2007). Of the biodegradable polymers, the poly(lactic-co-glycolic acid) copolymers (PLA, PGA, and PLGA) have been widely utilized as the most promising biodegradable materials, which have also been reported to be the most safe polymers used in vivo successfully with no significant toxicity (Agnihotri and Vavia 2009; Athanasiou *et al.* 1996; Dong *et al.* 2006; Kobayashi *et al.* 1992).

In a study of small pigment epithelium-derived factor (PEDF) neuroprotective effects, peptides injected intravitreally as free peptides or delivered in PLGA nanospheres, were tested in retinal ischemic injury in C57BL/6 mice. This study presented that injection of PEDF peptide (alone or as PLGA-based nanospheres) showed protective effects. However, the PLGA-PEDF nanospheres resulted in longer-term protection of the retinal ganglion cell layer with no noticeable side effects at 7days, thus conferring higher clinical advantages for longer-term treatments of retinal diseases (Li *et al.* 2006).

Agnihotri and Vavia (2009) successfully loaded diclofenac sodium in PLGA nanosuspensions, which were applied to rabbit eye and examined with a modified Draize test. These polymeric nanoparticles seemed to be devoid of any irritant effect on cornea, iris, and conjunctiva. Further, higher decrease of the sodium arachidonate induced inflammation was obtained by means of PLGA nanoparticles incorporating flurbiprofen in the rabbit eye after topical instillation, thus indicating its usefulness for inhibition of ocular inflammation (Vega *et al.* 2006). Similarly, Dong and coworkers (2006) reported that the intravitreal implantation of the cyclosporin A loaded PLGA can effectively reduce the intraocular inflammation in rabbits with no toxicity. Further, the intravitreal injections of a suspension of polylactic acid micro/nanospheres containing 1% adriamycin/doxorubicin were reported to provide sustained, first-order release for approximately two weeks. Using microarray technology, we have examined the toxicogenomic potential of the PLGA-based nanoformulations using small arrays hosing 200 gene spots, as a result of which no significant gene expression changes were observed (our unpublished data). Fig. 3 shows scanning electron micrographs of PLGA.

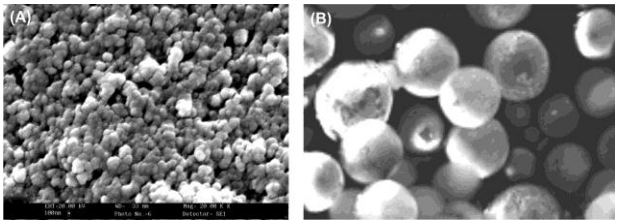

Fig. 3. Scanning electron micrographs of packed (A) and particulate single (B) PLGA.

Most of nanoparticles used for ocular investigations appeared to be mucoadhesive and biocompatible, nevertheless polystyrene (PS), Eudragit® RL100 (ERL) and RS100 (ERS) are not biodegradable. Fig. 4 shows

the scanning electron micrographs of the piroxicam nanoparticles formulated with ERS.

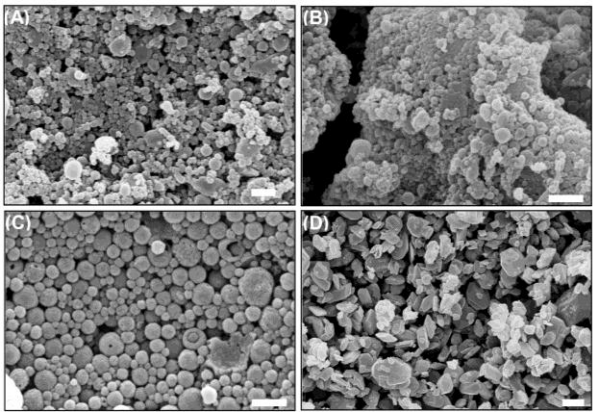

Fig. 4. Scanning electron micrographs of piroxicam formulations**.** A) Piroxicam:ERS nanoparticles at the ratios of 1:2.5. B) Piroxicam:ERS nanoparticles at the ratios of 1:10. C) Treated ERS. D) Treated piroxicam. Bar equals to 2 μm. ERS: Eudragit® RS100. The image was adopted with permission from (Adibkia *et al.* 2007b).

Chitosan, a deacetylated chitin, is biodegradable, biocompatible and nontoxic polymer, whose nanoparticles have been demonstrated to penetrate effectively conjunctival and corneal epithelial cells. It is a promising ophthalmic vehicle because of its probable superior mucoadhesiviness caused by electrostatic interactions with the negative charges of the mucosal layers.

In an interesting investigation, animals were treated with cyclosporine A-loaded chitosan nanoparticles, which resulted in significantly higher corneal and conj unctival drug levels than those treated with a suspension of cyclosporin A in a chitosan aqueous solution or in water (De Campos *et al.* 2001). It has also been demonstrated that the amounts of fluorescent nanoparticles in cornea and conjunctiva were significantly higher than that of a control solution. These amounts were fairly constant for up to 24 h. A higher retention of chitosan nanoparticles in the conjunctiva compared with in the cornea was observed (De Campos *et al.* 2004).

Liposomal nanomedicines

The vesicular lipid bilayers are basically defined as "liposomes", which can contain one or more aqueous compartments. Upon the number of bilayers, these lipid based globular structures can be categorized into multilamellar and unilamellar vesicles. The unilamellar vesicles include small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). Drugs, based on their solubility characteristics, can be entrapped in the lipid bilayers or the aqueous compartment (Fenwick and Cullis 2008).

Liposomal nanomedicines (LNM) were first developed to encapsulate small conventional therapeutic drugs, where the earliest attempts involved passive entrapment of drugs resulted in rapid production of stable, homogeneous populations of LUVs (~100 nm). Owing to the composition of LNMs, they are biodegradable and relatively nontoxic, which makes them interesting as drug-delivery systems. The cationic nanoliposomes have been evaluated for their genotoxicity potential in A431 and A549 cells, which resulted in significant gene expression changes mainly related to apoptosis signaling paths (Omidi *et al.* 2003b; Omidi *et al.* 2005).

Owing to the unique architecture of the nanoliposomes, when they used as an ocular drug delivery systems (DDS), the LNMs can come into intimate contact with corneal and conjunctival epithelial cells, facilitating drug absorption. The main goal of LNMs is to reduce side effects while maintaining or enhancing the efficacy of the administered medication. It should be noticed that the LNMs are not usually taken up by healthy tissue as is the free drug. The normal tissues in corneal/noncorneal routes are continuous, with non-fenestrated endothelium of the vasculature, and tight endothelial junctions (on the order of 5 nm) prevent the extravasation of small liposomal carriers. The basal tissues also inhibit the extravasation of macromolecules. Based upon the disease/drugs used, the LNMs can be used to passively target the designated markers, through which drugs could be accumulated selectively at sites of disease (Fenwick and Cullis 2008).

The impact of a single intravitreal injection of vasoactive intestinal peptide (VIP) loaded in rhodamine-conjugated liposomes (VIP-Rh-Lip) on experimental autoimmune uveoretinitis (EAU) has been investigated in Lewis rats. Clinical and histologic assessments showed that macrophages expressed transforming growth factor-beta2, low levels of major histocompatibility complex class II, and nitric oxide synthase-2 in VIP-Rh-Lip-treated eyes in which the intraocular levels of interleukin (IL)-2, interferon-gamma, IL-17, IL-4, GRO/KC, and CCL5 were reduced with increased IL-13. These findings clearly imply that the encapsulation of VIP within liposomes can effectively deliver VIP into the eye and prevent the EAU (Camelo *et al.* 2009). Elimination of liposomes from the vitreous occur via a diffusional process through the anterior chamber, where SUVs and LUVs show half-life of 10 and 20 days, respectively (Barza *et al.* 1987). Drug release from liposomal systems is dependent on the concentration of the drug in the liposome. Thus, in the case of long-term treatment, the high concentration of drugs encapsulated in liposomal carriers may raise problems associated with vitreous clouding; nevertheless these drawbacks may be

acceptable in endophthalmitis. Besides, sometimes liposome entrapment can decrease the efficacy of drugs as reported for amphotericin B in a rabbit model with fungal (Candida albicans) endophthalmitis (Barza *et al.* 1987). Despite huge investigations, at this stage, the liposomal drugs approved by the FDA are: liposomal daunorubicin (DaunoXome®, Gilead Sciences, Inc., approved in 1996); liposomal cytarabine (DepoCyt®, DepoTech Corporation, approved in 1999); liposomal Amphotericin B (AmBisone®, Fujisawa, approved in 1997); liposomal doxorubicin HCl (Doxil®, ALZA Pharmaceuticals, approved in 2007). Still, nano-scaled formulations are under investigations for ocular use.

Of the lipid based nanoformulations, the cationic lipids (CLs) have been widely used as gene delivery systems. These structures were initially exploited by Felgner *et al.* (1987), who used liposomes consisting of N-[1-(2,3-dioleyloxy) propyl]- N,N,N-trimethylammonium chloride (DOTMA) and dioleoylphosphatidylethanolamine (DOPE) for DNA traverse across cell membranes, and showed high level expression of the encoded gene. Numbers of novel cationic lipids have soon after been synthesized and shown to possess similar transfection activity within target cells. Cationic lipids possess either mono- or poly-cationic head groups. DOTMA, dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide (DMRI) and dioleoyloxy-3-(trimethylammonio) propane (DOTAP) are monocationic. While, Dioctadecylamidoglicylspermin (DOGS), N-(1-(2,3-dioleyloxy)propyl)-N-2-(sperminecarboxamido)ethyl)-N,N-dimethyl- ammonium trifluoracetate (DOSPA) and 3beta-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol) have polycationic head groups. DOGS (transfectam or lipofectin) and DOTMA are examples of mostly used CLs for transfection; reader is directed to see (Liu *et al.* 2003; Nicolazzi *et al.* 2003). Cationic lipid-based delivery systems possess positively charged surface, at which these lipid based nanosystems can attach the cell surface that normally display negative charges. The cellular toxicity of cationic lipids is deemed to be attributed with the surface charge potential of the cationic lipids. It should be evoked that the lipid-DNA lipoplex is thought to enter cells via adsorptive endocytosis and, by mechanisms not fully understood as yet, release nucleic acids out of the endosomal/lysosomal compartments with the net effect of yielding high uptake and intracellular delivery of genes and oligonucleotides (Pedroso de Lima *et al.* 2001).

Nanostructured dendrimers

Tomalia *et al.* (1984) developed the first dendrimer, which was named the Starburst™ polyamidoamine (PAMAM) dendrimer due to its dendritic branches and controlled starburst growth. This macromolecule is built on an ammonia core with extending branches of alternating methyl acrylate and ethylene diamine molecules (Tomalia *et al.* 1984). Fig. 5 represents the chemical structures of PAMAM (generation 3).

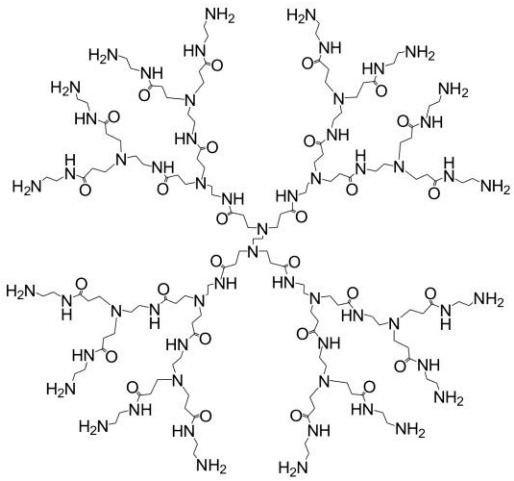

Fig. 5. Chemical structures of PAMAM (generation 3).

Dendrimers are composed of concentric, geometrically progressive layers created through radial amplification from a single, central initiator core molecule containing either three or four reactive sites such as ammonia or ethylene diamine. These nano-scale macromolecules are three dimensional and highly branched monodispersed nanostructures that are obtained by an iterative sequence of reaction steps producing a precise, unique branching structure (Loutsch *et al.* 2003).

Fig. 6 represents the chemical structures of polypropylenimine (PPI) diaminobutane (DAB) dendrimers; i.e., generation 2 (panel A) with 8 protonable surface amine groups and generation 3 (panel B) with 16 protonable surface amine groups.

These nanostructures provide globular nanosystems of 1-100 nm depending on the molecular weight and number of generations. Its surface ultimately determines the structure's interactions with its environment, as a result of which drugs/genes can be incorporated with and released in a controlled manner (Vandervoort and Ludwig 2007).

Interestingly, the influence of a controlled incremental increase in size, molecular weight and number of amine, carboxylate and hydroxyl surface groups in several series of PAMAM dendrimers for controlled ocular drug delivery were investigated. The duration of residence time for various generations (1.5, 2-3.5 and 4) in the New Zealand albino rabbit resulted in longer residence time for the solutions containing dendrimers with carboxylic and hydroxyl surface groups, which was

largely dependent on size and molecular weight (Vandamme and Brobeck 2005).

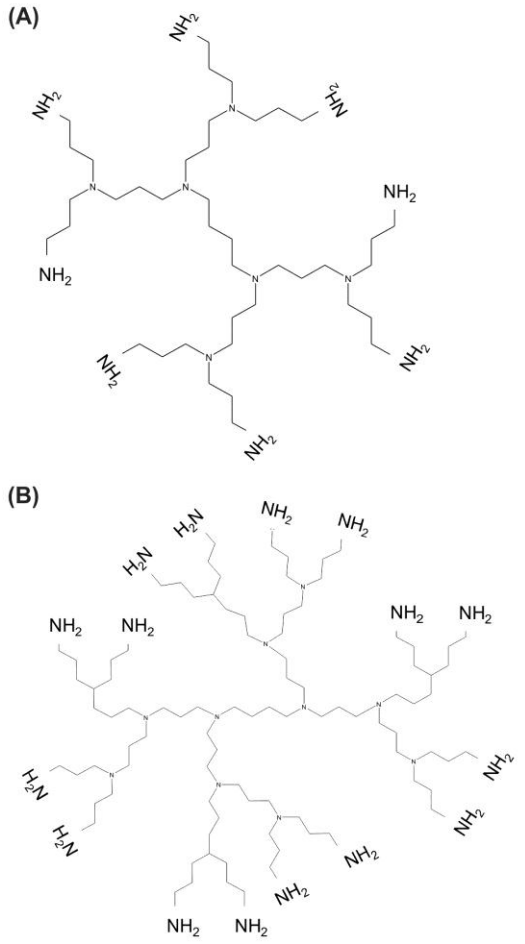

Fig. 6. Chemical structures of polypropylenimine (PPI) diaminobutane (DAB) dendrimers. A) DAB generation 2 with 8 protonable surface amine groups. B) DAB generation 3 with 16 protonable surface amine groups.

The modification of the dendrimer surface (e.g., addition of functional groups) is achievable through the addition of either subnanoscopic (e.g., small molecules) or nanoscaled reactants (e.g., DNA, antibodies, and proteins). The latter appears to be the preferred approach. For example, in a study to inhibit the laser-induced choroidal neovascularization (CNV), lipophilic amino-acid dendrimer was exploited to deliver an anti-vascular endothelial growth factor (VEGF) oligonucleotide (ODN) into the eyes of rats. Analysis of fluorescein angiograms of laser photocoagulated eyes revealed that dendrimer plus ODN significantly inhibited the development of CNV for 4-6 months by up to 95% in the initial stages, while ODN alone showed no significant difference (Marano *et al.* 2005).

Interestingly, generation 2 polypropyleneimine octaamine dendrimers crosslinked with collagen were reported to support human corneal epithelial cell growth and adhesion, with no cell toxicity. Thus, these nanostructures might be suitable scaffolds for corneal tissue engineering (Duan and Sheardown 2006). In ocular gene therapy, the control of gene transfection within the eye is merely an important issue, in particular when a light-induced delivery of DNA, drugs or other biological factors is the main objective. In a study, Nishiyama *et al*, (2005) devised a ternary complex composed of a core containing DNA packaged with cationic peptides and enveloped in the anionic dendrimer phthalocyanine (with a photosensitizing action). They showed that the ternary complex was able to profoundly (100-fold) enhance transgene expression in vitro with reduced photocytotoxicity, in which subconjuctival injection of the ternary complex followed by laser irradiation resulted in transgene expression only in the laser-irradiated site. This, surely, is a new biomedical application for dendrimeric nanostructures with successful results in the photochemical-internalization-mediated gene delivery in vivo (Nishiyama *et al.* 2005).

Nanomedicines paradigms in ocular diseases

In some diseases of the eye such as diabetic retinopathy, central retinal vein occlusion, choroidal neovascularisation (CNV) and intraocular solid tumors, angiogenesis play a key role, thus targeting the biomarkers within the ocular tissue is deemed to be an efficient treatment modality (Sahoo *et al.* 2008). Further, explicitly, no lymph system is presented in the retina environment. Thus, in retinal diseases attributed with neovascularization (e.g. wet AMD), treatment modes could be similar to the strategies which are recruited against solid tumors, i.e. displaying enhanced permeability and retention (EPR) effects. These facts further highlights the biological impacts on required pharmacotherapy to achieve enhanced drug permeation, controlled release of drugs, and targeted pharmacotherapy through specific targeting markers. The biological characteristics of the eye render this organ exquisitely impervious to the foreign substances, thus, for attainment of an optimal concentration at the intended ocular tissue of action through circumventing the ocular barriers, colloidal nanoparticle drug carriers have been devoted a great deal of attention (Bu *et al.* 2007).

Emergence of nano-scaled pharmaceuticals like nanosuspensions, solid lipid nanoparticles and liposomes appear to resolve the solubility-related problems of poorly soluble drugs such as piroxicam, dexamethasone, methylprednisolone, budenoside, gancyclovir (Kayser *et al.* 2005). Based upon the biological architecture of the eye together with the physicochemical characteristics of

the nanostructured medicines (i.e., particle charge, surface properties and relative hydrophobicity), these medications can be designed to successfully circumvent the blood-eye barriers. Since encapsulation of drugs can grant further protection as well as prolonged/controlled release, thus they confer better controlling tools for some chronic ocular diseases like chronic CMV retinitis, in which the intravitreal delivery of ganciclovir (GCV) seems to be the preferred strategy. Given its 13 h half-life, frequent injections of GCV is necessary to maintain therapeutic levels, however its use may be limited due to the consequential side effects such as cataract development, retinal detachment and endophthalmitis (Jabs 1995). Thus, to avoid repeated injections, intravitreal implants can be used to provide prolonged drug release even though some drawbacks like astigmatism and vitreous hemorrhage as well as couple of surgery requirements may limit its use, too (Muccioli and Belfort, Jr. 2000). These difficulties can be overcomed by using nanomedicines made up of various natural/biodegradable polymers like albumin and PLGA, because of their smaller size and controlled release properties (Sahoo *et al.* 2008).

Piloplex®, consisting of pilocarpine ionically bound to poly (methyl) methacrylate–co-acrylic acid, is a nano-scaled colloidal carrier system effectively used in glaucoma patients as twice-daily instillations. Multidimensional mechanisms appear to be involved for the pharmacologic action of ocular nanosystems including extending the time of drug residency in the cornea/conjunctiva, sustaining drug release from its carrier, reducing the precorneal drug loss and targeting the desired biomarker (Bu *et al.* 2007; Sahoo *et al.* 2008; Vandervoort and Ludwig 2007). Thus, it is highly desirable to exploit bioadhesive materials for formulation of nanosystems to be retained in the cul-de-sac after topical administration.

Various biodegradable and non-biodegradable carriers have been used, e.g. poly(lactic acid), PLGA, chitosan, poly(isobutyl cyanoacrylate) and Eudragit RS100 or RL100 (Bu *et al.* 2007). Erodible nanosystems are superior because the self-eroding process of the hydrolyzable polymer exert less harm on tissue (Herrero-Vanrell and Refojo 2001; Jose Alonso 2004). For example, PLGA colloidal nanoparticles were exploited to deliver gene-based therapeutics to the retinal pigment epithelial cells (Bejjani *et al.* 2005). The sustained-release nanosuspension of piroxicam and methylprednisolone acetate were formulated using Eudragit to control the endotoxin-induced uveitis in rabbits (Adibkia *et al.* 2007a; Adibkia *et al.* 2007b). For treatment of chronic ocular diseases (e.g. CMV retinitis), localized prolonged nanomedicines can be effectively used as a safer alternative of the frequent injections that may cause cataract development, retinal detachment, endophthalmitis and vitreous hemorrhage (Sahoo *et al.* 2008).

Nanosuspensions in ocular inflammation

The steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are routinely used in ocular surgeries, even though they often impose some adverse reactions. These medications are the most studied drugs to be exploited as ocular nanomedicines. Accordingly, localized therapy of ocular inflammation by these pharmaceuticals need to be optimized since most ocular diseases are classically treated with topical eye-drops which usually require frequent utilization of highly concentrated solutions. Enormous efforts, thus, have so far been devoted to maximize the localized delivery and targeting of desired pharmaceuticals using hydrogels, micro- and/or nanoparticles and liposomal formulations. We have previously reported that nanosuspension of piroxicam can control the endotoxin-induced uveitis (EU) in rabbits (Adibkia *et al.* 2007b), where cationic polymer (i.e. Eudragit®RS100) was used to formulate nanosuspensions of piroxicam by means of solvent evaporation/extraction technique (the also called single emulsion technique).

Given that the Eudragit®RS100 possesses an appropriate stability and size distribution characteristics together with its positive surface charge of about 30 mV, it is considered as a suitable ocular DDS (Pignatello *et al.* 2002a). The positively charged nanoformulations may interact with anionic mucins presented in the tear film, and cause consequential prolongation of drug residency time on the corneal surface (Dillen *et al.* 2006). Besides, the nanosuspensions may also confer more comfortableness for and better acceptance by patients in comparison with the routine ophthalmic suspensions that are basically formulated in micrometer ranges and show poor characteristics (Zimmer and Kreuter 1995). The ERL nanoparticles containing cloricromene (a coumarine derivative with antithrombotic and anti-ischemic activities) with positive zeta potential values (+27.3 mV) and a particle size of 80 nm were topically applied to rabbit eyes and showed no sign of toxicity or irritation to ocular tissues. A sustained release was observed in vitro as well as in vivo, resulting in a doubled AUC compared with an aqueous solution (Bucolo *et al.* 2004).

Fig. 7 represents in vitro release profiles of piroxicam (P) Eudragit®RS100 nanoparticles.

Overall, all nanoparticles showed a prolonged release profile without burst effect (Fig. 4), in which the complete release of drug after 24 hr (obeyed from Higuchi diffusion controlled model kinetics) explicitly indicate that there exists a structural homogeneity of the polymeric matrix, and also a more uniform distribution

of the drug. Modeling of drug release from nanoparticles of ciprofloxacin:Eudragit® has also been described by Dillen (2006), whose work showed that the release rate data fitted to the Higuchi's kinetic model. Based on our findings, treatment with piroxicam nanosuspensions significantly reduced observational symptoms of uveitis (based on Hogan's classification method) such as redness, presence of fibrin, photophobia, and lacrimation. We assume that the prolonged impacts of piroxicam nanosuspensions may be due to its interaction with local cellular components because of the positive surface charge of the nanoparticles in addition to the greater penetration and cellular uptake (Pignatello *et al.* 2002a).

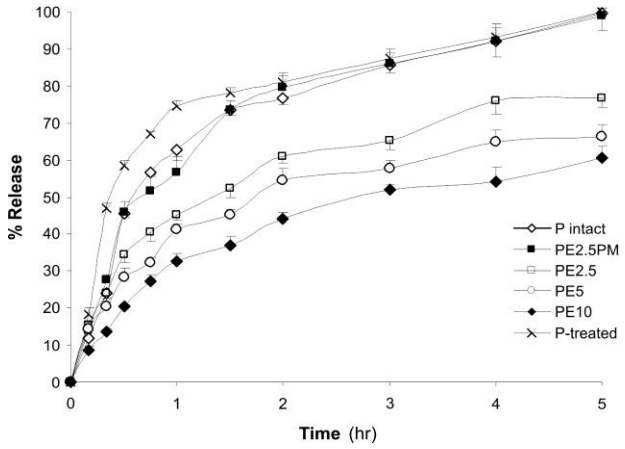

Fig. 7. In vitro drug release profiles. P-intact and P-treated represent the intact and treated piroxicam, respectively. PE2.5 indicates the piroxicam:Eudragit®RS100 nanoparticles at the ratios of 1:2.5. PM stands for physical mixture. Data represent mean value of 3-4 replications ± SE. The image was adopted with permission from (Adibkia *et al.* 2007b).

Given the cellular responses to the lipopolysaccharide (component of gram-negative bacterial cell wall) induced uveitis (Koizumi *et al.* 2003; Marie *et al.* 1999), it can be assumed that the piroxicam nanosuspensions perhaps favor the cellular recovery from EU by conferring a better therapeutic effect because of increased cellular uptake and enhanced inhibitory mechanism on the expression of the inflammatory mediators. Similarly, we formulated nanosuspensions of methylprednisolone acetate (MPA) using ERS to pursue their impacts on the inhibition of inflammatory symptoms in rabbits with EU. We found that the utilization of MPA-ERS nanosuspensions confers a controlled ocular delivery of MPA (Adibkia *et al.* 2007a).

Although molecular biology aspects of such therapies for uveitis is yet to be mechanistically investigated, it appears that the application of these types of nanosuspensions as a non-invasive approach seems to be safer controlled ocular delivery of anti-inflammation agents for inhibition of the uveitis symptoms. Similar results have been reported for ibuprofen and flurbiprofen (Pignatello *et al.* 2002a; Pignatello *et al.* 2002b).

Artificial vesicles such as liposomes, niosomes and discomes have been successfully utilized as vehicle for the ophthalmic drugs (e.g. oligonucleotides, acetazolamide, pilocarpine HCI, cyclopentolate and timolol maleate) resulting in improved ocular bioavailability. Of these, positively charged nanostructures seem to be preferentially captured at the negatively charged corneal surface and slow down drug elimination by lacrimal flow (Kaur *et al.* 2004; Sahoo *et al.* 2008). Using the laser-targeted delivery (LTD), it is likely now to release and activate the encapsulated drug within the heat-sensitive liposomes injected intravenously (Asrani *et al.* 2006). By virtue of being encapsulated, the drug is confined into the liposomes and shielded from general metabolism, by which efficient pharmacological effects with minimal adverse reactions are expected.

Photodynamic therapy: implementation of nanosystems

Photodynamic therapy (PDT) with verteporfin for choroidal neovascularization associated with retinal pigment epithelium detachment AMD (Pece *et al.* 2007), and combination of PDT with aforementioned nanomedicines (Ju *et al.* 2008; Lazic and Gabric 2007) have revealed promising results. These medications are unable to completely cure AMD, but they significantly decelerate the progression of the lesion growth in a proportion of patients. Ocular gene therapy has reached clinical trials (e.g., for inherited retinal degeneration), which possibly mark the culmination of decades of investigations (Bainbridge and Ali 2008). The eye, as a valuable model system for gene therapy, is a unique highly compartmentalized organ for efficient delivery of small volumes of viral (e.g., adeno/lenti-viral vectors) (Auricchio *et al.* 2002; Auricchio 2003; Hamilton *et al.* 2006) or non-viral (e.g. PEGylated nanoliposomes and niosomes) (Bloquel *et al.* 2006; ndrieu-Soler *et al.* 2006; Peeters *et al.* 2005; Sanders *et al.* 2007) vectors. Among them, the PEGylated non-viral nucleic acid nanostructures prevent their interaction with undesired biomolecules and providepromising results (Sanders *et al.* 2007). Besides, recent significant progresses in the mapping and cloning of retinal disease genes have provided great potential for gene therapy in the eye, e.g., gene replacement in the inherited retinal degenerations (Leber's congenital amaurosis due to defects in the gene encoding the enzyme RPE65) (Bainbridge *et al.* 2006; Le *et al.* 2007). In 2005, Kataoka and his coworkers reported light-induced gene transfer from packaged DNA enveloped in a dendrimeric photosensitizer. For

efficient transfection, the endosomal escape of the polyplexes is the main obstacle. This can be resolved by use of polycationic systems that possess buffering capacity (the so-called proton sponge effect). Thus, to obtain efficient photochemical internalization (PCI), these researchers assumed that the control of subcellular localization of photosensitizers may be a key to the PCI-mediated gene delivery with reduced cytotoxicity. At which, they developed a light-responsive gene carrier based on a ternary complex of pDNA, cationic peptides and anionic dendrimer-based photosensitizers, "dendrimer phthalocyanine" (DPc). In their work, the core polyplex was formed from a quadruplicated cationic peptide (CP_4), where a peptide (CP_2: C $(YGRKKRRQRRRG)_2$) was dimerized through a disulphide linkage, and pDNA was mixed with the CP_4 peptide at a molar ratio of cationic amino acids to a phosphate anion in DNA (i.e., N/P ratio of 2).

Using a luciferase (Luc) reporter gene assay in HeLa cells, they showed the transfection efficiency and cytotoxicity of the pDNA/CP_4 polyplex and pDNA/CP_4/DPc ternary complexes with varying charge ratios of DPc after irradiation of the light with increasing fluence. For in vivo PCI-mediated gene delivery, they pursued the transfection of a reporter gene (a variant of yellow fluorescent proteins, Venus) to the conjunctival tissue in rat eyes on laser irradiation after subconjunctival injection of the ternary complex. The pDNA/CP_4/DPc ternary complex with a charge ratio of 1:2:1 achieved significant gene expression only at the laser irradiated site in the conjunctiva 2 days after irradiation. This is a clear example of emergence of nanosystems towards futuristic use in PDT.

Genonanomedicines, monoclonal antibodies and nanobodies

In Sept. 2006, the global bio-nanotech company pSivida announced the initiation of a phase II clinical trial of Mifepristone as an eye drop treatment for steroid associated elevated intraocular pressure (see http://www.psivida.com/default.asp), for formulation of which a nanocarrier has possibly been used. More recently, a branched PEGylated anti VEGF aptamer (pegaptanib sodium marketed as Macugen®) was approved by the FDA for the treatment of neovascular AMD, which demonstrated the first oligonucleotide aptamer nanomedicine. It suppresses the pathological angiogenesis in the neovascular AMD by specifically targeting the extracellular VEGF resulting in inhibition of angiogenesis, reduction of permeability of the vascular bed and diminution of inflammation (Bakri and Kaiser 2006).

Further, ranibizumab is a recombinant humanized monoclonal antibody fragment (marketed as Lucentis®) that targets VEGF-A, an important mediator in the development of choroidal neovascularization, and reduces neovascularization and leakage in the wet AMD (Bakri and Kaiser 2006). Ranibizumab (48 kDa) is a markedly smaller molecule than RhuMAb VEGF (bevacizumab, Avastin®, 148 kDa) that is in early clinical testing for treatment of the choroidal neovascularization via intravitreal route (Bakri and Kaiser 2006). Unlike RhuMAb VEGF, the ranibizumab is able to penetrate the retina and enter the subretinal space after intravitreal injection because of its notable size difference.

The heavy-chain-antibodies (HCAbs) have recently been discovered in the blood of camelids. Because of their nano-scaled size (diameter of ~2.5 nm and height of ~4 nm), the antigen-binding units of these HCAbs comprising only a single Ig fold (see Fig. 8). Thus, they are called "Nanobodies®", whose several remarkable characteristics (i.e. being small, non-immunogenic, very stable, highly soluble, and easy to produce in large quantities) make them ideal candidates as next-generation immunotherapies. Antigen-specific Nanobodies® can easily be derived from the V_{HH} of HCAbs that are circulating in the serum of immunized llamas or camels.

Nanobodies® appear to be inherently soluble and stable, which usually do not aggregate and possess high homology with human V_H frameworks. Besides, they can be further humanized for use as therapeutics since these humanized nanostructured HCAbs are able to retain their characteristics and were shown to induce minimum immunogenicity (Muyldermans *et al.* 2009).

It should be evoked that the Nanobodies® can also be derived from the V_H domains of conventional antibodies, at which humanized Nanobodies® (the process that also called camelization) can be achieved through substitutions of specific amino-acid to improve these unstable V_H domains to become more stable with higher solubility. In fact, the single-domain nature of HCAbs confer several unique features in comparison with conventional Abs, although the conventional Abs show various beneficial characteristics including higher affinity and selectivity for a target, Nanobodies® display additional characteristics that make them superior as potential drug molecules. To our best knowledge, surprisingly, no studies have been conducted to use these unique structures for ocular targeting, but it is anticipated that they are not far from putting in practice.

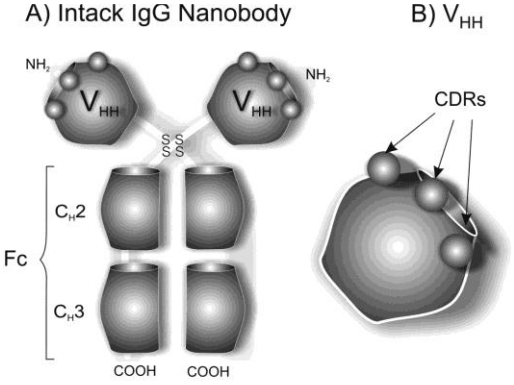

Fig. 8. Schematic structure of heavy-chain-antibodies (HCAbs). The image was adopted with permission from (Majidi *et al.* 2009).

Bioavailability of ocular nanomedicines

A variety of nanoparticle carriers undergo cellular uptake into ocular tissues via endocytosis. However, their characterization is limited to a qualitative basis only. The uptake percentage of the total dose nanoparticles and its contribution to overall ocular drug bioavailability remain unknown. In addition, an ophthalmic drug applied to the eye is subjected to metabolism when the drug penetrates across BEB into the site of action. In fact, there exist many researches demonstrating the functional expression of various enzymes involved in a variety of stages of drug metabolism and detoxification (Duvvuri *et al.* 2004; Rose and Bode 1991). Of these drug-metabolizing enzymes, oxidoreductases (e.g., aldehyde oxidase, ketone reductase, cyclooxygenase, monoamine oxidase and P450), hydrolases (e.g., aminopeptidase, acetylcholinesterase, carboxylesterase, aryl sulfatase, P-glucuronidase), and conjugating enzymes (e.g., arylamine acetyltransferase and glutathione S-transferase); for review see (Attar *et al.* 2005; Bu *et al.* 2007; Duvvuri *et al.* 2004). These metabolizing machieries of the eye are primarily expressed in various tissues (e.g., the retina-choroid), which appear to play an important role in ocular homeostasis by preventing entry of xenobiotics into, and/or eliminating xenobiotics from, the ocular tissues. Various cytochrome P450 (CYP) enzymes have been identified in ocular tissues including CYPs lA, 1B1, 2B, 2C, 2J, 3A, 4B1, 39A1, and NADPHreductase (Attar *et al.* 2005). Ocular nanomedicines loom to optimize the ocular bioavailability, for example a single topical instillation of acyclovir-PLA nanospheres in rabbits following resulted in significantly higher drug levels compared to the free drug formulation and exhibited a sustained acyclovir release for up to 6 h in aqueous humor (Giannavola *et al.* 2003). Kassem *et al.* (2007) evaluated the effect of particle size in the micron and nano-size ranges as well as the effect of viscosity of the nanosuspension on the ocular bioavailability of glucocorticoid drugs (hydrocortisone, prednisolone and dexamethasone) by measuring the intraocular pressure of normotensive Albino rabbits. They showed the nanosuspensions always enhance the rate and extent of ophthalmic drug absorption as well as the intensity of drug action. This clearly highlights higher bioavailability of nanosuspensions in comparison with micro-crystalline (Kassem *et al.* 2007). Recently, to provide long-term extraocular drug delivery using CS polymer, cyclosporin A (CyA) was formulated as nanoparticle with CS using an ionic gelation technique. The CyA-CS nanoparticles yielded mean size of 293 nm with zeta potential of +37 mV. In vitro release studies revealed prolonged drug release for a 24 h period. In vivo tests showed that, following topical instillation of CyA-CS nanoparticles to rabbits, therapeutic concentration was obtained in cornea and conjunctiva during at least 48 h, where the levels were significantly higher than those obtained following instillation of a CS solution containing CyA and an aqueous CyA suspension (De Campos *et al.* 2001). Very recently, to improve the precorneal residence time and the ocular bioavailability of indomethacin (IM), Badawi *et al.* (2008) deveoloped chitosan based nanoparticles (280 nm) and nanoemulsion (220-690 nm) using ionic gelation and spontaneous emulsification techniques, respectively. In vivo studies on eyes of rabbits displayed clearer healing of corneal by nanoemulsion, while CS nanocarriers were able to contact intimately with the cornea providing slow gradual IM release with long-term drug level thereby increasing delivery to both external and internal ocular tissues (Badawi *et al.* 2008). These findings support similar previous results (Calvo *et al.* 1996), in which suspensions of nanoparticles and nanocapsules made of poly-epsilon-caprolactone (PECL) yielded profound increased ocular bioavailability of indomethacin in rabbits eyes. Similarly, enhanced bioavailability was reported for topical use of nanoparticles of amikacin-poly(butyl cyanoacrylate) (PBCA), acyclovir-poly(ethyl cyanoacrylate), betaxolol-poly(isobutyl cyanoacrylate), cloricromene-ERL, cyclophosphamide-

PBCA, hydrocortisone-BSA, ibuprofen-ERS/ERL, metipranolol-PIBCA/PCL, progesterone-PBCA; for more details reader is directed to see (Bu *et al.* 2007). These all animal models based works are clear evidences for impacts of nano-scaled medicaments in ocular therapy despite their medical practices requires clinical trials.

Future prospective of ocular therapies

In ocular drug therapy, the need for safe application of medications to the posterior segment is deemed to be even more important than the surface delivery.

Treatment of intricate posterior segment diseases crucially necessitates safe drug delivery to the retina, the choroid, or the ciliary body. Systemic delivery and devices inserted into the vitreous are valuable strategies, so are the biodegradable/nonbiodegradable controlled-release implants inserted into both aqueous and vitreous. Moreover, in recent years, there has been a dramatic increase in understanding of the pathobiology of ocular diseases at cellular/molecular level. There exists now a large number of drugs under/in development (Frank 2003). For ocular drug therapy, this state of high flux resulted in few advanced therapeutics such as Visudyne®, Macugen® and the angiostatic anecortave acetate (Retaane®) which is administered as periocular injection every six months (Bakri and Kaiser 2006; Hayek *et al.* 2007).

In close proximity, it is also predictable to perceive nano-scaled technologies in practice, providing promising platform for improved non-invasive ocular drug delivery. However, further developments need to be accomplished to render the nanosystems more effective. The primary practical approach to provide nanomedicines with the necessary site adherence and site retention to achieve carrier and drug targeting in topical ocular therapy is to endow them with the ability to be a bioadhesive system, perhaps by utilization the natural biopolymers such as hyaluronic acid. The mutual use of penetration enhancers along with nanomedicines without compromising the stability of the system could also provide higher ocular bioavailability. The bioadhesive nanosystems can maximize ocular drug absorption by prolonging drug residence time in the cornea and conjunctiva and minimize precorneal drug loss, resulting in increased patient compliance. For development of the ocular bioadhesive systems, as localized sustained released medications, nonbiodegradable systems appear to be adequate to treat perforations and ulcerations. Ideally for long-term use, however, these systems should be nontoxic biodegradable adhesives with site specificity and minimal immunogenicity, yet improving bioavailability by enhancing absorption (particularly for protein/peptide based macromolecules) or inhibiting the metabolizing enzymes.

Based on the unique bioarchitecture of the eye, it is considered as a perfect organ for gene therapy because the delivery vector can rarely escape to the systemic sites. To date, the ocular pathologies have been tackled with 17 trials (phase I/II) focused on different conditions including retinitis pigmentosa, glaucoma, diabetic macular edema and AMD, while totally 1537 gene therapy clinical trials are in development; for more details see the following website (http://www.wiley.co.uk/genmed/clinical/). This highlights the growing interests in gene therapy of the ocular diseases, for which futuristic genomedicines are deemed to become more effective therapeutics by exploiting molecular Trojan delivery systems for safe shuttling of genomedicines (e.g. antisense, ribozyme and siRNA) and targeting the desired biomarkers (Janoria *et al.* 2007; Maguire and Bennett 2006). There is much excitement about the potential of the short interfering RNA (siRNA), which has remarkably rapidly moved towards applications. At this stage, 9 clinical trials are being developed for its implementation and most of these trials are involved in the ocular disease: 1) a phase I trial on "Cand5 anti-VEGF RNAi evaluation", which was started in 2004 by Acuity Pharmaceuticals, 2) a phase II trial on "Cand5 anti-VEGF RNAi evaluation (CARE) trial" which was started in 2005 by Acuity Pharmaceuticals, 3) a phase II trial on "RNAi assessment of cand5 in diabetic macular edema (RACE) trial" which was started in 2006 by Acuity Pharmaceuticals, 4) a phase I trail on "Open-label, dose-escalation single dose trial with Ssrna-027 in patients with AMD", which was in 2005 by Allergan Inc., 5) a phase II trial on "intravitreal injections of a siRNA in patients with AMD" targeting the vascular endothelial growth factor receptor-1 (Sirna-27) which was started in 2006 by Allergan Inc.

The LTD and PDT seem to be promising methodologies to deliver and to activate therapeutic and diagnostic agents to the retina and choroid. However, their successful applications largely depend on the appropriateness of the agent. Perhaps, combination of these techniques with gene therapy could benefit the ocular diseases. The encapsulated cell technology (ECT) and cell therapy appear to grant treatment potentials for the ocular diseases. ECT implants consist of living cells encapsulated within a semipermeable polymer membrane and supportive matrices, which are genetically engineered to produce a specific therapeutic substance to target a specific disease or condition. Once implanted, it allows the outward passage of the therapeutic product (Tao *et al.* 2006). It is anticipated that the biological properties of the eye would undergo the desired alterations through application of these technologies. However, for implementation of the cell therapy technology in human eyes, the validation of the technique will be a critical step. Besides, the cellular and subcellular/molecular aspects of the target tissues should be fully addressed and the ocular disease related biomarkers should be exclusively clarified. Possibly, high throughput screening technologies (e.g., DNA/protein array and phage display screening methodologies) would facilitate investigations towards specific targeting.

Finally, it should be stated that not all attempts to apply de novo nanotechnology approaches in biomedical sciences have met with the same success as those cited here in this review, and sometimes these novel

technologies tools provoke a great deal of challenges and hurdles. In fact, the nanostructures appear not to function in the same predictive ways that routinely used small molecules act, although this field is experiencing a rapid growth period with major advances in numerous diverse ways. Current preclinical investigations seem to provide new approaches to diagnose disease, to deliver specific therapy, and to monitor the biological impacts deeply. Although such fast inauguration of methodological alterations may eventually literally convey new challenges in the regulatory processes, it may grant a prolific platform from which will emerge many exciting, and yet unimagined, applications of biomedical nanotechnology.

Ethical issues

None to be declared.

Conflict of interests

Authors declare no conflict of interests.

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