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# Current and future ophthalmic drug delivery systems
# A shift to the posterior segment

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**Topical eye drop administration is useful only for the treatment of anterior segment diseases. The posterior eye segment is an important therapeutic target with unmet medical needs. The leading causes of visual impairment in the industrial countries are related to the disorders in the posterior eye tissues. New drugs for the medication of the posterior ocular segment have emerged, but most drugs are delivered by repeated intravitreal injections. Effective, safe, and comfortable methods of drug delivery are needed. The emerging methods include polymeric-controlled release injections and implants, nanoparticulates, microencapsulated cells, iontophoresis, and gene medicines. The biggest drug delivery challenge is to develop effective methods for posterior segment therapies that would also be applicable for the out-patient use.**

## Introduction

Posterior segment ocular diseases are the most prevalent causes of visual impairment in the industrial countries. These diseases include, for example, age-related macular degeneration and diabetic retinopathy. However, the ocular drug market is dominated by anterior segment drug therapies (e.g. antibiotics, anti-inflammatory agents, diagnostics, and intraocular pressure decreasing anti-glaucoma drugs), typically in eye drop formulations.

The rapid progress of the biosciences opens new possibilities to meet the needs of the posterior segment treatments. The examples include the antisense and aptamer drugs for the treatment of cytomegalovirus (CMV) retinitis and age-related macular degeneration, respectively, and the monoclonal antibodies for the treatment of the age-related macular degeneration. These compounds are given as intravitreal injections to the patients. Other new approaches for the treatment of macular degeneration and inherited retinal degenerations include intravitreal small interfering RNA (siRNA) and gene therapy, respectively.

This review will give an update and the recent progress and trends in ocular drug delivery systems.

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## Ocular pharmacokinetics: barriers in drug delivery

The ocular tissues can be reached either by local or systemic drug administration. The tissue barriers limit the access of drugs to their targets [1] (Figure 1). The corneal and conjunctival epithelial barriers cover the ocular surface. The blood–aqueous barrier, composed of the uveal capillary endothelia and ciliary epithelia, limits the access of compounds from the systemic circulation to the anterior chamber, whereas the blood–retina barrier limits the drug diffusion from the systemic blood to the retina and vice versa. The barrier has two components: outer and inner blood–retina barriers that are formed by the retinal pigment epithelium (RPE) and the tight retinal capillary walls, respectively.

After topical eye drop administration only less than 5% of the dose is absorbed into the eye [2]. The dose is mostly absorbed to the systemic blood circulation via the conjunctival and nasal blood vessels. For example, at least 70% of the timolol dose is systemically absorbed within 5 min [2,3]. Ocular absorption is limited by the corneal epithelium, and it is only modestly increased by prolonged ocular contact. Owing to the extensive conjunctival systemic absorption the maximal attainable ocular absorption is only about 10% of the dose [2].

Eye drops are used only for the treatment of the anterior segment disorders, since adequate drug concentrations are not reached in the posterior tissues using this drug delivery method.

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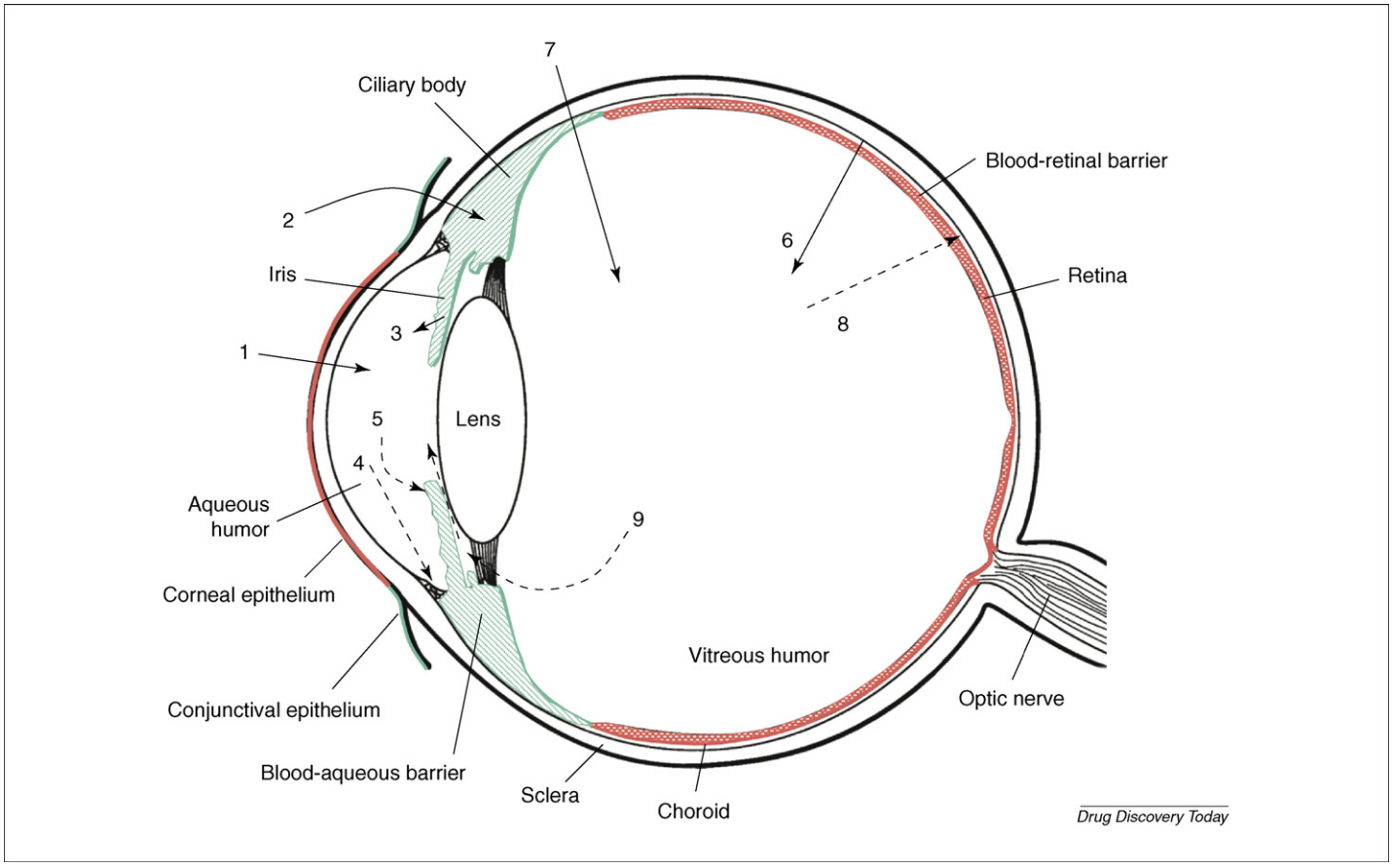



**FIGURE 1**

Schematic presentation of the ocular structure with the routes of drug kinetics illustrated. The numbers refer to following processes: (1) trans-corneal permeation from the lacrimal fluid into the anterior chamber, (2) non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea, (3) drug distribution from the blood stream via blood–aqueous barrier into the anterior chamber, (4) elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal, (5) drug elimination from the aqueous humor into the systemic uveoscleral circulation, (6) drug distribution from the blood into the posterior eye across the blood–retina barrier, (7) intravitreal drug administration, (8) drug elimination from the vitreous via posterior route across the blood retina barrier, and (9) drug elimination from the vitreous via anterior route to the posterior chamber [1].

The drug concentration difference between the cornea and retina is several orders of magnitude [2]. After administration in eye drops, most clinically used drugs are absorbed across the cornea to the anterior chamber (Figure 1). The non-corneal route, via conjunctiva and sclera, avoids the counter flow of aqueous humor and the lens barrier. Therefore, this route may be a viable future alternative for the administration of drugs (including proteins and gene-based drugs) to the retina and vitreous if appropriate delivery systems are developed. The sclera is permeable even to macromolecules, but choroidal blood flow and RPE are the major barriers in drug penetration [4,5].

Intravitreal injection can provide adequate drug concentrations in the posterior segment. Owing to the invasive nature of the injection, it is important to design drug formulations to maintain the therapeutic drug concentration over prolonged periods and minimize the number of injections. Drugs are eliminated via the anterior route, that is, to the aqueous humor and then eliminated by the outflow of the humor in the anterior chamber angle [6]. Many drugs are also eliminated posteriorly through the blood–retina barrier to the systemic circulation. Such drugs must have adequate permeability (i.e. lipophilicity or active transport) in the retinal capillaries and in the RPE [6]. In this case, the half-life of the drugs becomes short, even 2–3 h.

Finally, drugs may be delivered to the posterior ocular segment from the systemic blood circulation. Again, permeable drugs can cross the blood–retina barrier to reach the retina and vitreous [6]. Access to the choroid is easier owing to the extensive blood flow and leaky vessels in this tissue. Only a small fraction of the blood flow circulates through the posterior ocular segment. Therefore, high doses are needed and systemic adverse effects are common (e.g. systemic treatment of glaucoma with carbonic anhydrase inhibitors). Such approach is not feasible for potent drugs with narrow therapeutic indices. Localized targeting of such drugs to specific cells in the choroid, RPE, or retinal capillaries is an appealing approach.

## Evolution of ocular drug delivery systems

Eye drops have already been used at the times of Cleopatra for the treatment of ocular conditions. For example, *Belladonna* (i.e. atropine) was used as a mydriatic in ancient Egypt. The eye drops must be administered frequently, their ocular bioavailability is low (less than 5% of the dose is absorbed), and the posterior segment cannot be treated with them. For these reasons, prolonged action dosage forms with improved ocular absorption have been developed. The first polymeric inserts that release the drug over prolonged period were used already in the late 1800s in the U.K. These

gelatine inserts released cocaine for the purpose of local ocular anesthesia. Soluble ophthalmic drug inserts (SODI) were introduced in the 1960s in the Soviet Union. They were manufactured as several versions that contained drugs such as pilocarpine and mydriatics. The matrix of an acrylate-based co-polymer dissolved during a couple of hours after its application to the conjunctival sac. Pilocarpine releasing Ocusert® (Alza, USA) was introduced in the early 1970s in the Western world. This sophisticated system released the drug for a week at constant rate of 40 μg/h through ethylene vinyl acetate (EVA) membranes [7]. Later, another insert, Lacrisert® (Merck and Co. Inc., USA), was introduced for the treatment of dry eye syndrome [8]. These inserts did not become popular in the out-patient use because the elderly patients had difficulties to use them, and occasionally the insert was expelled from the conjunctival sac during sleep. This motivated the development of liquid state delivery system that forms a timolol releasing gel after its instillation as an eye drop [9]. Another product releases betaxolol to the tear fluid by ion-exchange from the surface of microspheres [10]. These approaches can modestly delay drug release and prolong drug action. Drug immersed hydrophilic contact lenses and topical ocular liposomes have been tested for drug delivery already in the 1970s and 1980s, but these approaches resulted only in limited improvements [11]. Importantly, the posterior ocular tissues cannot be treated with these topical ocular delivery methods.

The need to treat the posterior segment of the eye revived the interest in ocular-controlled release systems. Vitrasert® (Bausch & Lomb, USA), a polymeric ganciclovir implant, was introduced already in the 1990s for the treatment of opportunistic viral retinitis in the AIDS patients. Most research efforts are currently directed toward the dosage forms to treat the age-related macular degeneration and other retinal diseases.

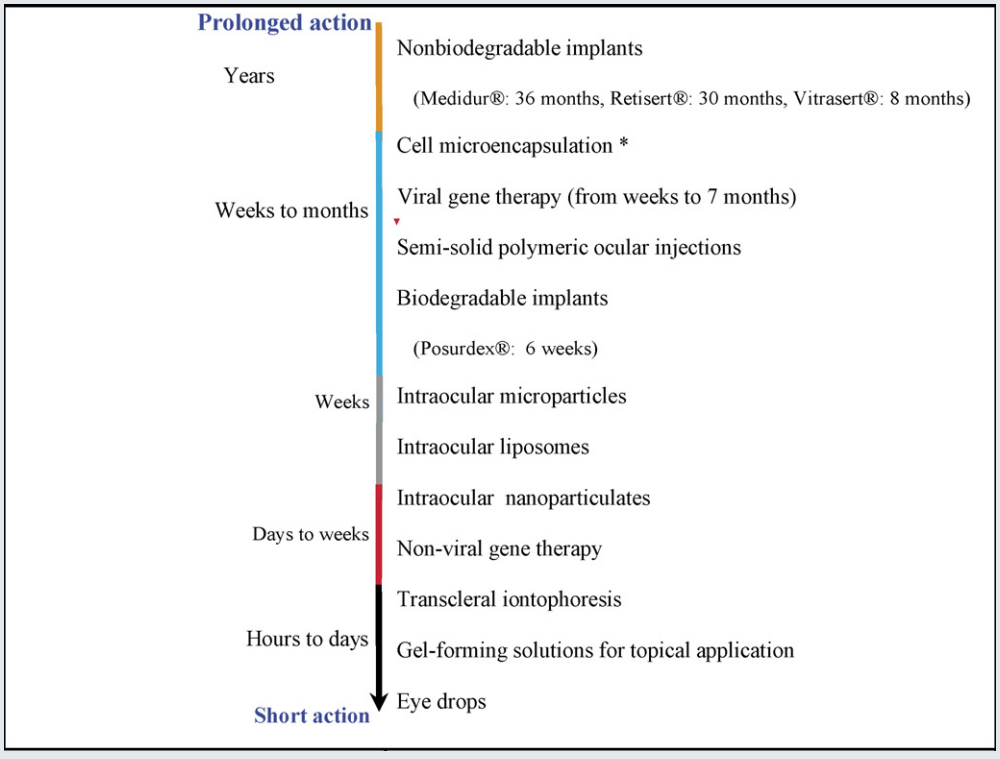
## Current status

Nowadays, there are, on the market or undergoing clinical trials, ocular drug delivery systems designed to sustain drug release. Most of them are for the treatment of long-term diseases that affect the posterior segment of the eye [12]. The important indications for ocular drug delivery systems include macular degeneration, viral infections (like CMV infections), glaucoma, ocular inflammations, dry eye syndrome, and retinal degenerations. There are many important indications for ocular drug delivery systems depending on the site of application and the delivered drug.

The challenge is to provide a system with improved ocular drug bioavailability and prolonged duration of activity (Box 1), but still with a minimum risk of ocular complications (Box 2). The mode of administration plays an important role in defining the safety of the device [13] (Table 1) and further, in achieving patient compliance and acceptance. In general the highest risk of ocular complications is with the invasive intraocular drug delivery. The risk is smaller with periocular drug administration. Ocular complications are rare for the systemic drug administration, but the systemic adverse effects are more prevalent.

**BOX 1**

### Duration of action of the ocular drug delivery systems




*To date, the only clinically tested formulation of microencapsulated cells has been implanted for six months in humans, and 18 months in rabbit eyes. Nevertheless, microencapsulated cells have potential for longer periods of implantation and action.

**BOX 2**

**Classification of the routes for ocular drug administration**

***Invasive drug administration to intraocular cavities***
- Intravitreal surgery (at the pars plana)
- Repeated[a] intravitreal injections
- Intracameral surgery (capsular bag)
- Subretinal injection
- Repeated[a] suprachoroidal injections
- Repeated[a] intracameral injections

***Invasive periocular and scleral modes of drug administration***
- Intrascleral surgery
- Episcleral surgery
- Repeated[a] periocular injections
- Repeated[a] subconjuctival injections
- Transscleral diffusion from controlled release systems

***Non-invasive methods***
- Topical administration on the eye

***Systemic administration***
- Intravenous infusion and injection
- Per oral

[a] The repetition is needed to accomplish long-term ocular treatment.

## Recent developments and future challenges in the field

### *Implants*

The goal of the intraocular implant design is to provide prolonged activity with controlled drug release from the polymeric implant material. Intraocular administration of the implants always requires minor surgery. In general, they are placed intravitreally, at the pars plana of the eye (posterior to the lens and anterior to the retina) [14–16]. Although this is an invasive technique, the implants have the benefit of (1) by-passing the blood–ocular barriers to deliver constant therapeutic levels of drug directly to the site of action, (2) avoidance of the side effects associated with frequent systemic and intravitreal injections, and (3) smaller quantity of drug needed during the treatment. The ocular implants are classified as non-biodegradable and biodegradable devices [14]. Non-biodegradable implants can provide more accurate control of drug release and longer release periods than the biodegradable polymers do, but the non-biodegradable systems require surgical implant removal with the associated risks (Table 1). The ocular implants are summarized in Table 2.

Vitrasert® [14] and Retisert® [16] (Bausch & Lomb, USA) are clinically used non-biodegradable implants. Vitrasert® is the first implantable ganciclovir delivery device (approved by the FDA in 1996). The EVA and polyvinyl alcohol (PVA) polymers control the release of ganciclovir. It is effective in controlling the progression of CMV retinitis associated with AIDS for eight months, but occasional endophthalmitis and increased rate of retinal detachments have been associated with this implant [14].

Retisert® [16] is the first marketed fluocinolone acetonide implant for the treatment of chronic non-infectious uveitis of the posterior segment. PVA and silicone laminate govern the release of the corticosteroid during three years. Although the device is effective in controlling the uveitis, it also presents side effects like cataract and increased intraocular pressure.

Medidur® (Alimera Sciences, USA and pSivida Inc., USA) implant (http://www.alimerasciences.com/medidur_overview.asp) can be inserted intravitreally as an injection, instead of a surgery. This tube-shaped device is in Phase III clinical trial for diabetic macular edema using fluocinolone acetonide. The device is very small (3 mm long and 0.37 mm of diameter) and its action lasts between 18 and 36 months after injection.

Kato *et al.* [17] carried out rabbit studies with scleral implant placed to the posterior pole. The device releases the drug, betamethasone, constantly for at least three months without detectable drug concentration in the aqueous humor. Interestingly, the implant showed more effective delivery to the macular region than the intravitreal implants. The episcleral system is a promising means for the treatment of the retinal and choroidal diseases.

Surodex® [18] and Posurdex® (Allergan, USA) [19] are the biodegradable implants in clinical Phase III studies. They are nearly identical poly lactic-co-glycolic acid (PLGA) implants with different doses of dexamethasone (60 μg for Surodex® and 700 μg for Posurdex ®). Surodex® was meant for the treatment of postoperative inflammation after filtering surgery on eyes with glaucoma placed underneath the scleral flap during the operation. Nevertheless, no further development of the implant is currently undertaken. Posurdex® is designed for the treatment of macular edema due to retinal vein occlusion, diabetic macular edema and uveitis by sustained release of dexamethasone over a month after intravitreal placement. In the future, Retisert® or Posurdex® may be chosen for the management of uveitis depending on the duration of the treatment needed.

Semi-solid bioerodible implant materials would enable delivery of soft implants with a needle and syringe. Heller [20] introduced such a material, poly(orthoester) IV, that shows long residence time after subconjunctival administration, an erosion-controlled drug release, and ocular biocompatibility. Depending on the ocular site of injection, the ocular lifetime of the drug ranges from five weeks to six months.

### *Microspheres*

Another controlled release strategy is to encapsulate the drug in the microparticles [21–25] (1–1000 μm) or nanoparticles [21,26–31] (1–1000 nm). Biodegradable and biocompatible polymers, such as polylactide and PLGA, both approved by FDA, are typically used. These systems are usually given as intravitreal injection, a less invasive procedure than the surgical implantation. They provide sustained drug delivery for weeks or even months [12,21] (Box 1), allowing reduced number of injections. Furthermore, these carriers can be engineered to target certain cell types. The intravitreal injections of the particulate systems may cause vitreal clouding [12]. However, microparticles tend to sink to the lower part of the vitreal cavity, while nanoparticles are more susceptible to cause clouding in the vitreous (Table 1).

To date, some microsphere formulations have been in preclinical studies, but have not yet undergone clinical trials. The microspheres were administered subconjunctivally or periocularly to provide trans-scleral drug delivery instead of the invasive intravitreal administration. Microspheres of PKC412 [22], inhibitor of protein kinase C and receptors for vascular endothelial growth factor (VEGF), were used to treat the choroidal neovascularization. After a periocular injection, PKC412 penetrated the sclera and

**TABLE 1**

**Advantages and disadvantages of the current and potential drug delivery systems to treat ocular diseases**

| | *Advantages* | Disadvantages |
|---|---|---|
| **Drops** | - Easy to apply<br>- The least invasive of the methods<br>- Good patient acceptance | - Poor ocular bioavailability<br>- Sometimes short duration of action<br>- Ineffective to treat diseases of the posterior segment of the eye<br>- The high concentrations or frequent instillations may lead to ocular and systemic toxicity<br>- Sometimes low patient compliance |
| **Systemic administration** | - More effective to treat diseases of the posterior segment of the eye than drops | - Most of the administered drugs do not by-pass blood–ocular barriers<br>- Side effects: systemic toxicity |
| **Intravitreal, periocular and subconjuctival injections** | - Improve drug absorption over systemically and topically delivered agents<br>- More safety drug delivery to the posterior segment of the eye than systemic administration (no systemic toxicity)<br>- Drug delivery to the target sites of the eye | - Injections display first-order kinetics (this rapid rise may cause difficulties with toxicity, and drug efficacy can diminish as the drug concentration falls below the targeted range)<br>- Injections have short half-life (few hours) and should be administered repeatedly<br>- Side effects: repeated injections can cause pain, discomfort, IOP increases, intraocular bleeding, increased chances for infection, and the possibility of retinal detachment; the major complication for intravitreal injection is endophtalmitis<br>- Poor acceptance by patients |
| **Implants** | - An alternative to repeated injections because they increase half-life of the drug and may help to minimize peak plasma level; they might improve patient acceptance and compliance<br>- Stabilization of the drug<br>- The non-biodegradable implants are more controllable delivery profile and longer periods of drug release than biodegradable ones<br>- The biodegradable implants do not need to be removed | - Side effects: the insertion of these devices is invasive and with associated ocular complications (retinal detachment and intravitreal hemorrhage for intravitreal implant)<br>- The non-biodegradable require surgery to harvest the device once is depleted of the drug (risk of ocular complications)<br>- The biodegradable implants have a final uncontrollable 'burst' in their drug release profile |
| **Microparticles, nanoparticles and liposomes** | - Stabilization of the drug<br>- Increase half-life of drugs (the frequency of injections diminishes)<br>- Decrease peak concentration resulting in decreasing the toxicity (micro and nanoparticles minimize 'BURST' in their drug delivery profile because the dose volume is limited)<br>- Localized delivery of drug (RPE cells)<br>- Improved patient compliance and convenience | - Side effects: risk associated with injections and vitreous clouding |
| **Cell encapsulation** | - Long-lasting and continuous expression of the given protein (avoiding repeated injections) without genetic alteration of the host tissues<br>- Delivery directly to the target site (limiting toxicity)<br>- Easy retrieval of the implant when desired (making the treatment reversible)<br>- Improve patient compliance | - Side effects: invasive method with the complications related to the surgical insertion and removal<br>- Patient acceptance to be seen |
| **Iontophoresis** | - Non-invasive method and easy to use<br>- May combine with other drug delivery systems<br>- Ability of modulate dosage (less risk of toxicity)<br>- Good drug penetration to anterior and posterior segment of the eye<br>- Good acceptance by patients<br>- A broad applicability to deliver a broad range of drugs or genes to treat several ophthalmic diseases in the posterior segment of the eye | - No sustained half-life: requires repeated administrations<br>- Side effects: mild pain in some cases, but no risk of infections or ulcerations<br>- Risk of low patient compliance because the frequent administrations that may be needed |

significantly suppressed choroidal neovascularization. Gomes dos Santos *et al.* [23] designed PLGA microspheres for the sustained release of the nanosized anti-TGFβ2 (transforming growth factor β2) phosphorothioate antisense oligonucleotide complexes. A subconjunctival injection prevented post-surgical fibrosis following trabeculectomy for 42 days. Since periocular and subconjunctival injections are less invasive than the intravitreal injections (Box 2), these studies demonstrate the potential of trans-scleral

**TABLE 2**

**Description of current and potential ophthalmic implants**

| *Registered name* | Active substance | Mode of administration | Implant size | Marketing status |
|---|---|---|---|---|
| **Vitrasert®** | Ganciclovir | Surgical implantation at the pars plana | Millimetre size | Clinical use |
| **Retisert®** | Fluocinolone acetonide | Surgical implantation at the pars plana | Tablet 3 mm × 2mm × 5 mm | Clinical use |
| **Medidur®** | Fluocinolone acetonide | Injected in the vitreous cavity | Cylindrical tube 3.5 mm in length and 0.37 mm in diameter | Phase III |
| **Posurdex®** | Dexamethasone | Injected or through small incision at the pars plana | Microsized implant | Phase III |
| **Surodex®** | Dexamethasone | Placed underneath the scleral flap | Microsized implant | Phase III |
| **NT-501** | CNTF secreted by encapsulated cells | Surgical implantation at the pars plana | Cylindrical tube 6 mm in length and 1 mm in diameter | Phase II |

route for prolonged drug delivery. Furthermore, Carrasquillo *et al.* [24] used microsphere system to release pegaptanib sodium (anti-VEGF aptamer) continuously at the scleral surface to provide prolonged delivery of the released aptamer. The study is relevant in the context of subconjunctival or peribulbar oligonucleotide delivery into the eye. Recently, a second drug delivery system for pegaptanib sodium was described [25]: intravitreal PLGA microspheres released aptamer over several weeks after injection.

### *Nanoparticulates for drug delivery*

Many ocular nanoparticulate systems, like polymeric nanoparticles, liposomes, and micelles, are currently tested pre-clinically. Several systems are based on self-assembly of the surfactants, lipids, or polymers, while some nanoparticles require special processing.

## *Nanoparticles*

Pre-clinical experiments have demonstrated nanoparticulates in RPE cells [26–28]. This is probably due to the phagocytic capacity of RPE, and indicates that nanoparticulates could be used to treat the retinal disorders. Bourges *et al.* [26] showed that the nanoparticles are retained within the RPE cells even four months after a single intravitreal injection. Thus the method may provide steady and continuous delivery of drugs or oligonucleotides.

Albumin nanoparticles are an interesting delivery system for intravitreal drug administration that has shown controlled drug release and degradation to safe products [29]. *In vivo* rat studies demonstrated their localization in the vitreous cavity and ciliary body for at least two weeks after a single intravitreal injection [29].

## *Micelles*

Micelles are generated using amphiphilic surfactants or diblock polymers. Recently, a polyion complex micelle system that incorporates a dendritic phtalocyanine photosensitizer [32–34] was tested in rats for its efficacy in photodynamic therapy of choroidal neovascularization. The micellar system exhibited absorption at 650 nm, which is advantageous for the treatment of deep lesions. The formulation may prolong the retention in the blood circulation and achieve a selective accumulation in the choroidal neovascularizated lesions, but these aspects require further development.

## *Liposomes*

Liposomes are lipid vesicles of about 25–10 000 nm in diameter. There are various methods for preparation of liposomes with different sizes, stability, and pharmacokinetics. Liposomes [35] are biocompatible, biodegradable, and they can be made of natural lipids. Hydrophilic and lipophilic drugs can be encapsulated to the lipid walls or the aqueous interior of the liposomes, respectively. The liposomes are taken up by phagocytic cells, like the RPE cells thus enabling intracellular drug delivery. Further, the liposomal surfaces can be modified to allow preferential binding, for example, to the endothelia of proliferating neovascular vessels. The limitations include potential intraocular clouding after the intravitreal injections. Liposome technology has been used to develop light-induced systems for the retinal diseases (for example Visudyne®, Novartis Pharmaceuticals, USA) [36,37].

## *Light-induced systems*

Light-induced systems can be divided to two groups: light-activated drugs (i.e. photodynamic therapy) and light-activated drug delivery systems.

Verteporfin (Visudyne®) [36,37] is the only ocular liposomal drug currently in the clinical use. It works as photodynamic therapy to treat choroidal neovascularization and age-related macular degeneration. After intravenous infusion of Visudyne®, a non-thermal red laser is applied to the retina to activate verteporfin that causes local damage to neovascular endothelium, thus resulting in occlusion of the targeted vessels. Photodynamic therapy itself induces an increased local production of VEGF and potential reappearance of the choroidal neovessels [36]. Hence, the effect of Visudyne® is insufficient in some cases and the patients need repeated treatments.

Rostaporfin (Photrex®, Miravant Medical Technologies, USA) [37,38] is another liposomal photosensitizing agent that aims to treat age-related macular degeneration. Currently its FDA approval is pending. The frequency of the required treatments is significantly lower than Visudyne®.

A light-induced drug delivery system based on VP22, a structural protein of Herpes Simplex virus, was described recently [28]. The purified VP22 protein binds antisense oligonucleotide of human *c-raf* kinase. A fluorochrome is covalently linked to the protein or to the oligonucleotide. The spherical particles, vectosomes, migrated trans-retinally after an intravitreal injection into the rats. The carriers remained in the cytoplasm of various retinal cell types and trans-scleral illumination triggered the delivery of free oligonucleotides. The *c-raf* kinase plays a pivotal role in cell proliferation, resistance of apoptosis, and has influence on the VEGF

pathway. This is a promising method to treat neovascular tissue in the age-related macular degeneration and diabetic retinopathy.

Another light-triggered drug delivery system was described by Paasonen *et al.* [31]. They immobilized gold nanoparticles into the liposomes. Upon light exposure the liposomal contents were released, presumably owing to the heat transfer from the gold nanoparticles into the lipid bilayers, and subsequent increased leakiness of the lipid layer. The light-controlled drug delivery to the intraocular structures is of particular interest owing to the wide use of lasers in ophthalmology. Nevertheless, attention should be paid to the choice of the wavelength and energy of the laser to avoid retinal adverse reactions.

### *Non-viral delivery systems for gene-based drugs*

Gene-based drugs include gene therapy and other approaches that rely on the specific nucleotide sequences. The nucleotide sequence of DNA, RNA, or their modifications is used to induce gene expression (gene therapy), suppress translation of the target mRNA (siRNA, antisense oligonucleotides, ribozymes), or to bind to a specific protein target (aptamers) [39]. These approaches are applicable in the treatment of ocular diseases [40–42] and have some advantages compared with the conventional drugs. Firstly, compared with small molecule drug discovery it is easier to find the relevant nucleotide sequence of gene-based drug. Genes can express their protein products for prolonged periods and further control can be obtained by cell specific or inducible promoters [40].

Currently there are two ocular gene-based drugs in the clinical use. The first one was Vitravene®, fomivirsen sodium, intravitreal phosphorothioate oligonucleotide for the treatment of CMV infection in the AIDS patients [41]. The second one, pegaptanib sodium (Macugen®), is an anti-VEGF aptamer for the treatment of wet age-related macular degeneration [43,44]. The aptamer is conjugated to polyethylene glycol (PEG) to increase its half-life and stability in the vitreous. The same drug is currently in Phase II trials for the treatment of diabetic macular edema. In addition, two siRNA molecules (bevasiranib and Sirna-027) that modify the activity of VEGF and its receptor (VEGFR-1) have entered in the clinical trials (http://www.gene.com/gene/index.jsp, http://www.sirna.com). They are given as intravitreal injections.

In general, the gene-based medicines are hydrophilic large molecules with suboptimal delivery properties. Even though the compounds are administered in saline solution intravitreally, the delivery systems are useful in improving the intracellular access of these compounds and to improve their stability in biological fluids [41]. Thus, improvements in the efficacy and duration of action can be achieved. The delivery systems are classified into viral and non-viral vectors. Although viral vectors are more efficient in the delivery of genes, the non-viral systems have some advantages, such as the lack of immune response, the ease of formulation, and unlimited gene size [30]. In this drug delivery review we are describing only the non-viral delivery systems, because these technologies are applicable also in the delivery of aptamers, siRNA, and antisense compounds.

Some examples of pre-clinically investigated systems include microparticles [23–25], nanoparticles [26–31], and liposomes [45]. For example, a plasmid DNA compacted with PEG-substituted lysine peptides was used for non-viral gene transfer into the eye [30]. Other systems include polyamidoamine dendrimer (treelike branched macromolecules) [46–48] and a cationic lipophilic peptide [49]. They are investigated as carriers to deliver antisense oligonucleotide of VEGF to treat choroidal neovascularization. One of the advantages of such systems is the prolonged drug activity after intravitreal injection. Bochot *et al.* [45] show that after intravitreal injection, the liposome formulation protected the oligonucleotide from the degradation and prolonged its vitreal residence time. The liposomes also reduced the distribution of oligonucleotide to non-target sites. Preferential uptake by the retinal cells opens up interesting treatment perspectives.

Cationic lipids and polymers are frequently used owing to binding and condensing of DNA and RNA to small particulates in the range of 100 nm. Despite their small size the nanoparticulate systems have limited diffusion in the tissues. For example, vitreous and neural retinas are substantial barriers that prohibit the spreading of the injected nanoparticulate systems after intravitreal administration [50,51]. This may be due to the steric hindrance and electrostatic interactions with ocular polyanions, such as hyaluronic acid and chondroitin sulfate.

Recently, the corneal epithelial barrier for protein drug absorption was overcome by transfecting the surface epithelial cells. Toropainen *et al.* [52] used eye drops that contained plasmid DNA condensed with cationic lipid composition DOTAP/DOPE. After topical ocular instillation the surface layer of the cornea and conjunctiva were transfected with marker gene encoding secreted alkaline phosphatase (SEAP). SEAP was secreted from the cells into the tear fluid and anterior chamber for several days. Similarly, the differentiated RPE can be transfected *in vitro* with liposomal nanoparticulates (DOTAP/DOPE/protamine sulfate) to secrete the protein over prolonged periods of even two months [53]. These studies demonstrate the use of transfected ocular tissues as a platform for the secretion of therapeutic protein.

#### *Cell encapsulation*

The principle of encapsulated cell technology (ECT) [54,55] is to entrap immunologically isolated cells with microcapsules or hollow fibers before their administration into the eye. This technology enables the controlled, continuous, and long-term delivery of therapeutic proteins directly to the back of the eye. This allows the implantation of genetically engineered cells that continuously produce the therapeutic protein at the site of implantation. Small 6 mm long ECT implants are placed through a small incision in the pars plana of the eye, yet outside of the visual axis. The implant is sutured in a manner that allows its retrieval when desired.

ECT product NT501 (Neurotech Inc., USA) [54] has just completed Phase I trials and is now entering Phase II. One eye in each of 10 patients with retinitis pigmentosa was implanted with the device for six months. During that time, the polymer implant that contains genetically modified human RPE cells secreted ciliary neurotrophic factor into the vitreous of the patients' eyes. The device proved to be well tolerated by patients and showed an improvement in visual acuity scores of some patients. ECT could potentially serve as a delivery system for chronic ophthalmic diseases without effective therapies (e.g. neuroprotection in glaucoma, anti-angiogenesis in choroidal neovascularization, anti-inflammatory factors for uveitis) [55]. However, its long-term safety and efficacy remains to be seen.

### *Iontophoresis*

Iontophoresis is a non-invasive method of transferring ionized drugs through membranes with low electrical current [56,57]. The drugs are moved across the membranes by two mechanisms: migration and electro-osmosis.

Ocular iontophoresis is classified into trans-corneal, corneoscleral or trans-scleral iontophoresis [56], the latter being the most interesting option. The sclera has larger surface area than the cornea (about 17 $cm^2$ versus 1.3 $cm^2$) high degree of hydration, low number of cells, and it is permeable to large molecular weight compounds [4]. Trans-scleral delivery allows drug transfer to the posterior segment.

A few ocular iontophoresis systems have been investigated recently: Ocuphor® (Iomed Inc., USA) [58], Eyegate II Delivery System® (EyeGate Pharma, USA) [59], and Visulex® (Aciont Inc., USA) [60–63]. These devices avoid the adverse effects that were frequently observed in the past studies with higher electrical current densities [58,59]. These devices are also easier to use than the older iontophoretic systems. Eyegate II Delivery System® [59] is the first one that was used in the patients. On the basis of the first experiences it is effective, easy to use, and well tolerated.

The iontophoretic technique is less invasive than the intraocular injections, but the duration of the drug action is less prolonged than with the controlled release systems. Unlike transdermal iontophoresis the mechanisms of the trans-scleral iontophoresis have not been investigated in detail. However, the properties of the sclera (hydration, leakiness, low electrical resistance) make the iontophoretic delivery feasible at low electric currents or voltages. This has been shown in the case of corticoids, immunosuppressive agents, and oligonucleotides [56,59–64]. The drug loss by the conjunctival and choroidal blood flow was decreased by co-administration of a vasoconstrictor (oxymethazoline) [63]. The efficacy and patient acceptance in the broad clinical applications are not yet known.

### *Gelifying systems*

Another strategy to obtain an ocular sustained drug delivery system is to use various phase changing polymers. These materials can be delivered in a liquid form, as an eye drop or intraocular injection. After instillation the polymer undergoes a phase change and forms a semi-solid or solid matrix that releases the drug over prolonged period. The phase transition can be induced by the change in the temperature, ion concentration, or pH. There are several polymers that have such properties.

For topical ocular use, the gel forming solutions, such as Timoptic-XE® (Merck and Co. Inc., USA) (http://www.merck.-com/product/usa/pi_circulars/t/timoptic/timoptic_xe_pi.pdf), Pilogel® (Alcon, Inc., Switzerland) (http://home.intekom.com/pharm/alcon/pilogel.html) and Azasite® (Insite Vision, USA) (http://www.insitevision.com/marketed_products) are already in the clinical use. For example, Timoptic-XE® contains gelrite (purified anionic heteropolysaccharide from gellan gum), whereas poly(acrylic acid) is incorporated in the Pilogel® eye drops. These materials enhance the drug retention relative to the conventional eye drops and lead to increased drug absorption into the eye and reduced dosing frequency. However, the duration of drug activity can be increased by hours, not by days or weeks. It has been assumed that the gelifying systems could improve out-patient compliance, but this was not the case in a recent study described by Kahook [65].

Clearly, a new breakthrough is needed before topical ocular gelifying delivery systems can provide truly prolonged drug action or drug delivery to the posterior segment. Subconjunctival and parabulbar gelifying injections are interesting alternatives for the posterior segment drug delivery, but their final therapeutic value must be further investigated.

## Conclusions

Ocular drug delivery has become an increasingly important field of research. Advances in the ocular drug delivery systems research are expected to provide new tools for the treatment of the eye diseases with the new therapeutic modalities, like antibodies, aptamers, and siRNA. These delivery methods should provide prolonged action, less invasive administration, higher efficacy, and improved safety. The injectable or implantable prolonged acting systems should guarantee patient adherence to the drug therapy. Development of sustained and non-invasive posterior segment delivery systems for the self-administration by the out-patients is still an unrealistic technological challenge. Major technological breakthroughs are needed before that goal becomes reality. There are several scientific and technological advances that are driving the progress in this field. Especially the advances in nanotechnology and biomaterials science may provide new smart technologies to augment ophthalmic drug delivery.

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