Application of lipid nanoparticles to ocular drug delivery

Application of lipid nanoparticles to ocular drug delivery

Luigi Battaglia*[1], Loredana Serpe[1], Federica Foglietta[1], Elisabetta Muntoni[1], Marina Gallarate[1], Ana Del Pozo Rodriguez[2], Marian Solinis[2]

1: Università degli Studi di Torino, Dipartimento di Scienza e Tecnologia del Farmaco – via Pietro Giuria 9 – Torino, Italy

2: Pharmacokinetic, Nanotechnology and Gene Therapy Group (PharmaNanoGene), Faculty of Pharmacy, Centro de investigación Lascaray ikergunea, University of the Basque Country UPV/EHU, Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain

Acknowledgements

This work was supported by the Basque Government's Department of Education, Universities and Investigation (IT-341-10) and by the Spanish Ministry of Economy and Competitiveness (SAF2014-53092-R). The authors would also thank Ricerca Locale 2014 (Italian MIUR) for funding.

Abstract

Introduction

Although eye drops are widespread as drug delivery systems for the anterior segment of the eye, they are also associated with poor drug bioavailability due to transient contact time and rapid washout by tearing. Moreover, effective drug delivery to the posterior segment of the eye is challenging, and alternative routes of administration (periocular and intravitreal) are generally needed, the blood retinal barrier being the major obstacle to systemic drug delivery.

Areas covered in this review

Nanotechnology, and especially lipid nanoparticles, can improve the therapeutic efficiency, compliance and safety of ocular drugs, administered via different routes, to both the anterior and posterior segment of the eye.

This review highlights the main ocular barriers to drug delivery, as well as the commonest eye diseases suitable for pharmacological treatment in which lipid nanoparticles have proved efficacious as alternative delivery systems.

Expert opinion

Lipid-based nanocarriers are among the most biocompatible and versatile means for ocular delivery. Mucoadhesion with consequent increase in pre-corneal retention time, and enhanced permeation due to cellular uptake by corneal epithelial cells, are the essential goals for topical LN delivery. Gene delivery to the retina has shown very promising results after intravitreal administration of lipid nanoparticles as non-viral vectors.

1. Introduction

Eye drops have been globally accepted as a formulation for anterior segment applications, although they are also associated with some limitations in terms of desired pharmacological and pharmacokinetic profile, dosing frequency, systemic untoward effects, patient non-compliance, low drug bioavailability due to transient contact time, rapid washout by tearing and lachrymal drainage [1]. In addition, conventional ocular formulations are generally unable to overcome the anatomical/physiological barriers between eye surface and the inner eye structures. Therefore, effective ocular drug delivery is a challenging proposition for the pharmaceutical scientist, and nanotechnology approaches can improve the therapeutic efficiency, compliance and safety of ocular drugs [2]. Lipid-based nanocarriers are among the newer and more interesting colloidal drug delivery systems; their extreme biodegradability and biocompatible chemical nature have secured them the title of 'nanosafe' carriers [3]. This review highlights the main ocular barriers for drug delivery, as well as the commonest eye diseases treated with lipid nanoparticles (LN) as alternative delivery systems.

2. Anatomy and physiology of the eye

The human eye possesses a well-defined anatomy divided into two regions (Figure 1): the anterior segment (cornea, conjunctiva, iris, ciliary body, the anterior and posterior chambers, the lens, the lachrymal apparatus and the eyelids) and the posterior segment (sclera, choroid, retina, and vitreous) [4, 5, 6, 7, 8].

The cornea is the clear transparent outer layer of the eye. It is a non-vascularized structure composed of three layers, namely epithelium, stroma, and endothelium, separated respectively by the Bowman's and Descemet's membranes. The region where the cornea and the sclera converge is covered by the conjunctiva, a thin vascularized mucus membrane with a clear transparent surface in which are located the goblet cells responsible for the mucus secretion. This secretion constitutes the innermost layer of the pre-corneal tear film that covers the eye surface [9]. The tear film has also an

outermost layer composed of a mixture of lipids secreted by the Meibomian glands in the eyelids [10], and an aqueous middle layer that consists of a salt solution of a wide variety of proteins secreted predominantly by the lachrymal gland [11, 12].

The anterior and posterior chambers in the anterior segment are filled with aqueous humor, which is responsible for the intraocular pressure (IOP) [13]. The anterior chamber is bounded in front by the cornea and a small portion of the sclera, and behind by the iris, the lens and a part of the ciliary body. The posterior chamber is limited by the iris and the lens.

In the posterior segment of the eye, the sclera is an opaque fibrous protective layer, slightly elastic, with a composition similar to that of the cornea, although the structural organization of the collagen fibres differs. Scleral collagen has wider fibrils and a much more interwoven structure than the cornea. Sclera maintains IOP and serves as the attachment site for the extraocular muscles, which maintain eye shape during ocular movement [14, 15] The retina and the lens surround the vitreous, in which is located the vitreous humor, a transparent gelatinous fluid necessary to let the light reach the retina and to maintain the shape of the eye [16].

3. Routes and barriers of ocular drug delivery

The blood ocular barriers (BOB) are highly specialized and selectively control the inward/toward traverse of compounds. They are the main obstacles in the systemic treatment of intra-ocular disorders. The two main sites of the BOB are [17]:

i) the blood aqueous barrier (BAB), located in the anterior part of the eye, which is composed of endothelial cells of blood vessels in the iris and the non-pigmented ciliary epithelium.

ii) the blood retinal barrier (BRB), located in the posterior part of the eye. The BRB is composed of two types of cells, i.e. the retinal capillary endothelial cells and the RPE cells, which constitute the inner and the outer BRB, respectively [18, 19]. The tight

junctions of the RPE and the inner BRB slow down the drug's penetration from the blood into the posterior segment of the eye after systemic administration.

The permeability of the BOB also varies depending on the drug's characteristics. It is higher for lipophilic drugs, since they are permeable through retinal capillaries and through the RPE; however, the blood flow to the posterior segment of the eye is limited [20]. Consequently, high doses are necessary, and there is a significant risk of adverse effects, thus hampering the use of drugs with a narrow therapeutic range [21]. Corticosteroids, immunosuppressive agents, and antibiotics are examples of orally administered drugs for the treatment of posterior segment eye diseases [22], but alternative ocular drug delivery systems or administration routes are now being considered [23, 24].

There are several possible routes for drug delivery into the ocular tissues, depending on the target tissue (Figure 1).

3.1. Anterior segment of the eye

For the management of diseases in the anterior segment of the eye, topical administration is by far the commonest route. Drug transport via corneal/non-corneal routes involves several intricate biological processes; consequently, the bioavailability of topically applied drugs is poor in the internal tissues of the eye [17].

The main barriers for ocular drug delivery are [25]:

i) Elimination from lachrymal fluid (pre-corneal barrier): most of the instilled volume is either drained from the conjunctival sac into the naso-lachrymal duct or cleared from pre-corneal area, resulting in poor bioavailability of drugs.

ii) Corneal barrier: anatomically, the corneal barrier is due mainly to intercellular tight junctions (*zonula occludens*) which completely surround the superficial epithelial cells, serving as a selective barrier for small molecules and completely preventing the diffusion of macromolecules via the paracellular route. Corneal stroma, instead, is a highly hydrophilic tissue with an open structure that allows the diffusion of hydrophilic

drugs up to 500 kDa size, while it is a rate-limiting barrier for most lipophilic drugs. The corneal endothelium is responsible for maintaining normal corneal hydration, and it has been estimated that drugs with molecular dimensions up to about 20 nm can diffuse.

The drug transport across the corneal epithelium is essentially via paracellular or transcellular routes. The hydrophilic drugs penetrate primarily through the paracellular pathway, while lipophilic drugs prefer the transcellular route. Lipophilicity, solubility, molecular size, charge and degree of ionization also affect the route and rate of penetration in the cornea [26].

Particulate material in the nanometer range has been reported to follow the endocytic pathway depending on the optimized lipophilic-hydrophilic properties of the matrix and on reduced particle size [27].

iii) Non-corneal absorption: topically applied ocular drugs may be absorbed through the bulbar conjunctiva and the underlying sclera into the uveal tract and vitreous humor, which results in drug loss at desired site.

Moreover, it should be considered that the drug reaching the anterior eye chamber can bind to melanin pigments in the iris and ciliary body, reducing its bioavailability. As regards drug pharmacokinetics, it is also influenced by drug metabolism in ocular tissues mediated by enzymes such as esterases and cytochrome P-450 reductase [28].

In some particular cases, intracameral injections are used for direct drug delivery to the anterior chamber (e.g. acetylcholine [29]). However, general anesthesia is necessary for administration, and physical damage to intraocular structures, such as the corneal endothelium, iris and lens, may be associated with intracameral injections [26].

3.2 Posterior segment of the eye

Unfortunately, topical administration achieves negligible or very low drug levels in the retina and vitreous humor. Among the alternative routes, intravitreal injection delivers drugs directly into the vitreous. Small molecules, usually below 500 Da, diffuse more rapidly than large ones. After intravitreal injection the administered drug is eliminated by two main routes, either anterior and/or posterior (via blood flow and aqueous turnover). Nonetheless, frequent administration of drugs via this route can lead to retinal detachment, retinal haemorrhage, endophthalmitis and increased IOP or cataract [30, 31]. To minimize some of these complications, novel drug-delivery systems have been developed, such as biodegradable or non-biodegradable implants, which can be placed long term in the vitreous [32].

Another possible route is subretinal injection. This route allows contact of the active molecules with the outer retina, and it is especially useful for retinal degenerations originating in photoreceptors and RPE. This invasive method is usually employed in gene therapy studies [33], although it is associated with ocular damage (i.e. lesions in RPE, hemorrhages, retinal tears, sub- or pre-retinal fibrosis, and retinal detachment) [34].

The commonest route of instillation for posterior eye tissues is periocular administration, including subconjunctival and retrobulbar injections. This enables the deposition of molecules on the external surface of the sclera, thereby minimizing the risk of endophthalmitis and retinal damage associated with the intravitreal route of administration [25, 28]:

i) Subconjunctival route: the formulation is placed beneath the conjunctival membrane that covers the sclera. This enables the drugs to bypass the conjunctiva–cornea barrier, giving direct access to the transscleral route. The sclera is less resistant to permeation of molecules and has lower protease activity compared to the cornea.

ii) The retrobulbar route involves inserting a needle through the eyelid and orbital fascia and depositing a drug behind the globe into the retrobulbar space. Retrobulbar injection may sometimes damage orbital structures such as the optic nerve.

4. Commonest eye diseases suitable for pharmacological treatment

Ocular diseases range from minor troubles, such as conjunctivitis, to vision-threatening disorders which can affect both the anterior and posterior segments of the eye. The different diseases require suitable drug administration routes and drug delivery systems according to the target tissue. The intravitreal route is widely used to deliver drugs to the retina, even if it is associated with recurrent side effects [27, 35]. The topical route represents a safer administration, but it is limited to the anterior segment of the eye: moreover, because of poor corneal retention, only 1–10% of the instilled dose crosses this barrier and roughly 1% reaches the aqueous humor [27, 35]. The systemic route (e.g., oral, parenteral) is limited by the BOB [27, 35].

4.1 Corneal diseases

Different corneal diseases can potentially benefit from pharmacological treatments with LP, through topical administration [36].

i) *Graft rejection after keratoplasty:* immunosuppressant treatment is required after surgery to prevent the recurrence of graft rejection. Topical treatment with corticosteroids is currently the gold standard. Moreover, infections produced by Herpes Simplex Virus (HSV-1) are responsible for most cases of rejection of corneal grafts [37].

ii) *Dry eye syndrome* is a chronic and typically progressive condition. In most cases, it can be managed successfully, usually resulting in noticeably greater eye comfort, fewer dry eye symptoms, and sometimes sharper vision as well. Because dry eye disease can have a number of causes, a variety of therapeutic approaches are used, ranging from immunosuppressant drugs like Cyclosporine A (CsA), to artificial tears or anti-inflammatory treatments like steroid eye drops.

iii) *Corneal neovascularisation* is a sight-threatening condition usually associated with disorders of the ocular surface. Anti-Vascular Endothelial Growth Factor (VEGF) therapy, obtained both through corneal gene therapy and monoclonal antibody can

significantly decrease corneal neovascularization and lymphangiogenesis, resulting in increased graft survival in keratoplasty [38].

iv) *Wounds of the corneal epithelium*: Corneal wound healing is a complex process involving cell death, migration, proliferation, differentiation, and extracellular matrix remodelling. It can be treated with various approaches, including the use of trans-retinoic acid for corneal re-epithelization [39].

4.2 Glaucoma

Glaucoma is a multi-factorial optic neuropathy and is the second leading cause of blindness worldwide. A major risk factor is increased IOP in the eye, when the ratio between aqueous humor formation (inflow) and its outflow is unbalanced.

Lowering IOP via various pharmaceuticals and/or surgical techniques is currently the mainstay of glaucoma treatment. The topical route is the one most commonly used, owing to its suitability for chronic administration. Drug bioavailability can be improved by the delivery system, also decreasing the dosage [40]. Treatment options are “inflow inhibitors” (beta-antagonists, carbonic anhydrase inhibitors) and “outflow enhancers” (alpha-agonists, prostaglandin analogs and miotic agents) [41]. Innovative “inflow” (hydroxysteroid dehydrogenase-1 inhibitors; melatonin) and “outflow” agents (dopamine, serotonine and adenosine agonists, cannabinoids) are currently under study [42, 43].

4.3 Retinal diseases

i) *Angiogenesis-related blindness (ARB)* indicates the spectrum of retinal disorders whose pathogenesis is related to pathologic angiogenesis, including age-related macular degeneration (AMD), diabetic retinopathy, and retinopathy of prematurity. All these conditions produce retinal and choroidal neovascularization, leading to visual loss. Conventional treatment options for ARB used to be surgery and focal treatment, such as

laser photocoagulation and cryotherapy. In recent years, intravitreal injection of anti-VEGF monoclonal antibodies has been widely performed to treat pathologic angiogenesis. Its main limitations are its relatively short durability, the difficulty of performing repeated injections, and the risk of aggravating retinal ischemia, inducing mitochondrial disruption of photoreceptor cells [44].

ii) *Retinal degeneration* occurs in inherited or genetic ocular diseases such as retinitis pigmentosa, Stargardt disease, Leber Congenital Amaurosis (LCA), X-linked juvenile retinoschisis (XLRS). Most of these disorders have no pharmacological therapy aside from the experimental use of antioxidants in some cases [44], and there is no definite cure treatment. Nowadays, due to their genetic origin, these diseases are studied as targets of gene therapy.

4.4 Ocular inflammation

Intraocular inflammation is a clinical disorder induced by various biological (bacteria, virus, fungus, or parasites), chemical or physical agents (as a result of injury to the eye). In some cases, ischemia, hypersensitivity, or autoimmunity causes may be involved. Postoperative inflammation is a common occurrence following ophthalmic surgical procedures, especially keratoplasty [17, 45].

Inflammation can affect several structures of the anterior and posterior segment of the eye, although the inflammation of the uveal tract (uveitis) is the commonest inflammatory disease of eye. It can be classified as anterior, intermediate, and posterior uveitis. Anterior uveitis (iritis, ciclitis) is the most frequent and has serious implications including glaucoma and cataract [17, 45] and is usually treated by the topical route.

Corticosteroids are the preferred choice for the treatment of ocular inflammation. The long-term side effects of corticosteroid include IOP, glaucoma and cataract [46]. Non steroidal anti-inflammatory drugs (NSAID) have mild anti-inflammatory activity compared with corticosteroids, and, additionally, some undesired effect on the eye, like irritation and burning sensation or

superficial punctuate keratitis [47]. Mydriatic/cycloplegic (anticholinergic) drugs are specifically used in the management of anterior uveitis because they minimize the pain by immobilizing the iris, preventing its adhesion to the anterior lens. Immunosuppressants (methotrexate and azathioprine) are used in patients resistant to corticosteroids [17, 45].

4.5 Ocular infectious diseases

Infections concerning the cornea (infective keratitis) and/or conjunctiva (infective kerato-conjuctivitis) are the most frequent. They are treated by topical route. For bacterial keratitis, broad-spectrum antibiotics demonstrating adequate coverage against both Gram-positive and Gram-negative pathogens are used (fluoroquinonolones, aminoglucosydes, chloramphenicol). Herpetic keratitis has a long history of safe treatment with acyclovir. Corneal fungal infections (Candida, aspergillus) are rare, but very serious: besides topical antifungal drug administration, addition of oral antifungal treatment is indicated in the case of deep corneal invasion and intraocular spread [48].

Endophthalmitis is a rare, but frequently devastating infection of the vitreous, caused mainly by bacteria, mycobacteria (tuberculosis) or fungi. Exogenous endophthalmitis is most frequently post-operative (keratoplasty); endogenous endophthalmitis, caused by the hematogenous spread of organisms from a remote infectious site into the eye, accounts for 2–15 % of all cases. The most important component of treatment is the intravitreal injection of antibiotics, along with vitrectomy in severe cases [49].

5. Animal and alternative models for ocular drug delivery systems

Corneal permeation of drugs has been extensively studied on animals, rabbits in particular. Drug pharmacokinetic in the aqueous humor, after topical administration in the lower conjunctival sac, can be followed either through anterior chamber paracentesis in anesthesized animals, or after animal sacrifice, which allows explantation of all other ocular tissues as well. Considering the

availability of quantifiable ocular responses *in vivo* (miosis, mydriasis, IOP, aqueous humor flow), pharmacological response measurements usually replace concentration assays in experimental studies [35]. Pre-corneal retention time, instead, can be evaluated, after topical administration, through withdrawal of the rabbit lachrymal fluid from the conjunctival sac by means of a capillary.

However, in recent years various models have been developed in order to perform ex vivo/in vitro permeation studies, limiting the use of animals: the most widely used are excised cornea tissues, usually from rabbits [50]. Alternative models include primary cultures of the cornea, mainly also from rabbits [51], reconstructed tissue cultures (three-dimensional cornea constructs from fetal porcine corneal cells, or completely engineered organotypic human cornea constructs) [51], immortalized cell lines from rabbit or human cornea and conjunctiva [52].

In order to test the corneal toxicity of ocular formulations, Draize's technique, performed in vivo on rabbits, is currently approved by the Food and Drug Administration (FDA). However, the need to limit the use of animal models in toxicity studies is leading towards the validation of alternative methods [53]. The ones most employed in experimental papers are the following:

i) *chorioallantoic membrane (CAM) test:* the CAM, that is the vascularised respiratory membrane surrounding a chick developing inside an egg, is incubated with the test formulation, and then changes in its morphology are scored

ii) *cornea opacity and permeability test (BCOP):* in this procedure, bovine corneas are explanted and quickly mounted on specially designed holders composed of two separate chambers. Corneal opacity and Na-fluorescein permeability through the cornea are measured as toxicity parameters after 10 minutes exposure to test formulations (EURL-ECVAM validated)

iii) *determination of corneal hydration levels* evaluated through determination of the corneal fresh and dry weight and compared to untreated corneas

iv) *commercial kits*: EYTEX™, EpiOcular™

As to retinal pharmacotherapy, immortalized human retinal pigment epithelial (ARPE-19) cells have been fully characterized regarding their morphology, the expression of retina-specific markers and their barrier properties, and they have been used for the development of targeted drug delivery systems to the posterior segment of the eye [52]. For animal experiments, the most used and widely accepted animals are rabbits [54]. Because retinal and choroidal neovascularization are the main mechanisms related to ARB, suitable animal models have been developed [55]. In the case of genetic retinal disorders, various animal models have been also generated [56, 57, 58, 59].

6. Colloidal carriers for ocular delivery

Colloidal carriers for topical administration are designed to be endocytosed by the corneal epithelial cells and act as a reservoir to release the drug slowly. These carriers prevent tear washout by providing a sustained release of ocular drugs. Moreover, they can inhibit the activity of P-gp expressed on epithelial cells [60], and open corneal tight junctions by means of non-ionic surfactants present in the formulations [12]. In the treatment of posterior segment diseases, they can act as controlled release systems that reduce administration frequency.

They usually contain particles smaller than 1000 nm. Nonetheless, 50 to 400 nm particles are better tolerated by patients than larger ones, because they are abler to penetrate across the corneal barrier [61].

Among colloidal carriers, vesicular systems (liposomes, niosomes), nanoemulsions, lipid and polymeric nanoparticles are the most promising for ophthalmic drug delivery. Table 1 summarizes the advantages and drawbacks of these carriers.

Biodegradable polymeric nanoparticles can be composed of various polymers in which the drug is dissolved, entrapped, encapsulated, or attached to the surface [61].

Nanoemulsions are fine dispersions of biocompatible oils droplets in the nanometer range, stabilized by high surfactant concentration. They restore both the lipid and water component of the tear film, thus reducing evaporative fluid loss and, owing to the emulsifiers, they have been found

to improve the ‘wettability’ of tear film [12]. However, in some cases this produces a sticky feel of the formulation and subsequent intolerance [60].

Liposomes are vesicular systems with diameters ranging from 50 nm to several microns and composed of natural biocompatible phospholipids [62]. Niosomes are non-ionic amphiphiles vesicular self-assemblies. Compared to other vesicular systems they are chemically very stable, biodegradable, biocompatible and non-immunogenic. [12] The vesicular membrane is flexible and supports deformation stress, allowing repeated intraocular injections to treat posterior segment ocular diseases [60].

7. Lipid nanoparticles (LN)

LN are particles in the 50-1000 nm range whose matrix is made of biocompatible solid lipids or mixtures of solid and liquid lipids.

Solid lipid nanoparticles (SLN) are considered to be among the most effective lipid-based colloidal vehicles. They are constituted by a solid lipid matrix surrounded by a layer of surfactants in an aqueous dispersion. The main SLN production methods are based on solidified nanoemulsion technologies, the most important of them being high pressure homogenization, melt-emulsification, and ultrasonication. Among the other best-known methods for SLN production, microemulsion templates, solvent-based methods, coacervation, supercritical fluid technology, and the membrane contactor method have been described [63].

Nanostructured lipid carriers (NLC) are LN characterized by a solid lipid core consisting of a mixture of solid and liquid lipids: the resulting matrix of the lipid particles shows a melting point depression compared with the original solid lipid, but the matrix is still solid at body temperature. Depending on the method of production and on the lipid blend composition, different types of NLCs are obtained: imperfect, amorphous and multiple type. In the imperfect type, lipid crystallization is altered by small amounts of oils. In the amorphous type, the lipid matrix is solid

but not crystalline (amorphous state): this can be achieved by mixing special lipids, for example, hydroxyoctacosanyl hydroxystearate with isopropyl myristate. In the multiple type, the solid lipid matrix contains tiny oil compartments: they are obtained by mixing a solid lipid with a higher amount of oil. The basic idea is that by altering the nanostructure of the lipid matrix, the payload for active compounds is increased and expulsion of the compound during storage is avoided [63]. (Figure 2)

Due to the lipidic core of SLN and NLC, the loading capacity of hydrophilic drugs is limited. To overcome this limitation, lipid-drug conjugates (LDC) nanoparticles have been developed. In a typical process, an insoluble drug-lipid conjugate bulk is first prepared either by salt formation (e.g., with a fatty acid) or by covalent linking (e.g., esters or ethers) [63]. The lipophilic conjugate may be used alone or processed in the same way as SLN or NLC [66].

So far, when compared to other colloidal systems, LN have been described as superior carriers, with various advantages [61, 64, 65], as mentioned in Table 1.

8. Application of LN in ocular delivery

Significant and recent experimental works have assessed the potential of LN as carriers for both the topical and alternative routes in the ocular delivery of different drugs commonly used for the most frequent ocular diseases (Table 2). Experimental studies have been compared in terms not only of the results obtained but also of the experimental models used, which can be related to their current stage of development.

8.1 Delivery to the anterior segment of the eye

LN suspensions are mainly conceived for topical administration, and consequently for the anterior segment of the eye. In these cases, the main objective for nanoparticulate formulation is to increase retention time at the corneal surface by muco-adhesion and improve corneal permeation through endocytic uptake by cornea epithelial cells.

Cationic LN obtained by using cationic lipid as surfactants [67, 68, 69, 70, 71] or functionalized with chitosan [72, 73, 74] are frequently used for this purpose, since they can interact with negatively charged mucus. Thiolated LN can be used to enhance muco-adhesion as well [75, 76]. Moreover, due to their physico-chemical stability, LN can be included in thermo-gelling medium in order to increase permanence of the formulation in contact with the cornea and avoid drainage from lachrymal fluid [77].

However, despite the biocompatibility of the lipid matrix for the topical application, a potential toxicity issue of LN should be considered: the surfactant used for the formulation of nanoparticles. A recent study, performed with a modified Draize test method, revealed a limit concentration for ocular tolerability of SLN stabilised with some specific surfactants, even if generally considered as GRAS [78].

8.1.1 Infectious disease

The delivery of drugs against infectious diseases in LN aims to enhance precorneal retention by increasing concentration in the biophase, compared to conventional eye drops. Moreover, LN should provide sustained drug release, so that the released drug can exert a suitable biocidal activity.

Different antibiotics commonly used for eye infectious diseases, such as tobramycin [79], chloramphenicol [80] gatifloxacine [81, 82, 83] or levofloxacine [84], have been loaded in LN, although the loading capacity was low compared to the antibiotic efficient dose. Ion paired tobramicyn [79] was entrapped in SLN and after topical administration to rabbits, ocular tobramycin bioavailability increased due to greater ocular residence time and entrapment of SLN in the mucin layer, owing to their small particle size (80 nm) and the lecithin surfactant employed, resulting in a sustained release of tobramycin. In other work [83], gatifloxacin loaded cationic SLN have shown promising corneal permeation on excised goat corneas, causing no significant effect on the corneal hydration level. SLN-entrapped levofloxacin [84] has exhibited cumulative amounts

permeated through excised cornea and maintained cornea hydration. Moreover, SLN has shown comparative antibacterial activity against *S. aureus* and *E. coli in vitro* with respect to marketed eye drops; however, *in vivo* studies for levofloxacin-SLN should be performed to determine its ophthalmic delivery efficacy.

Antifungal and antiviral drugs have also been encapsulated in LN. For example, itraconazole was successfully loaded in stearic acid and palmitic acid SLN [85]. Drug permeation, tested through an *ex-vivo* model of excised goat corneas, was higher from stearic acid SLN than from palmitic acid SLN. Ketoconazole [86] was successfully entrapped in the lipid core of SLN, providing enhanced permeation through cornea and higher bioavailability in the aqueous humor and also in the vitreous. The developed SLN were able to cross the ocular barrier, reach even the posterior segment of the eye, and had significant antifungal potential.

In another case, acyclovir loaded NLC and SLN were compared [87]. NLC showed higher encapsulation efficiency, superior physical properties, better release profile, and drug permeation was good only for NLC formulation. The oily component of the NLC probably plays an important role in drug encapsulation, with consequences on biological activity of nanoparticles.

8.1.2 Inflammatory diseases

Topical administration of LN is the preferred route to manage ocular inflammations of the anterior segment of the eye, precorneal retention and corneal-conjunctival uptake being the main mechanisms of action hypothesized. In some processes, i.e. those inducing a decrease of visual acuity or further complications (e.g. ocular surgery), rapidity in establishing the treatment is a crucial factor in avoiding inflammatory reaction and improving the patient's visual rehabilitation; consequently, sustained release of the drug from nanoparticles is a major requirement.

The majority of experimental works have dealt with NSAID, such as diclofenac [88], ibuprofen [67] , flurbiprofen [72, 89], indomethacin [90], even if one experimental work has considered corticosteroid triamcinolone acetonide [91].

Diclofenac sodium-loaded SLN were prepared by using goat fat templated with Phospholipon 90G® [88]. Phospholipids were essential, both in order to avoid drug burst release effect, and to increase drug ocular bioavailability, in a reconstructed cornea construct.

When ibuprofen was loaded in cationic NLC [67] the presence of stearylamine (cationic lipid) enhanced pre-ocular retention *in vivo* on rabbits; moreover, in *ex vivo* corneal permeation studies, different NLC formulations revealed significant permeation enhancement, compared to simple eye drops. Formulations containing Gelucire® 44/14 and Transcutol® P significantly enhanced penetration in rabbit excised cornea. Gelucires can enhance corneal penetration, mainly due to their lipophilic properties, whereas Transcutol® P enhances permeation by a mechanism involving structural changes in the epithelium as a result of micelle formation. All the tested formulations were not irritant *in vivo*.

In studies concerning flurbiprofen-loaded NLC [72], chitosan coating provided a longer pre-ocular retention time *in vivo* on rabbits, due to its mucoadhesive properties, and an improved penetration rate in excised rabbit cornea.

8.1.3 Oxidative stress

Antioxidants can potentially be used for the therapy of both the anterior and the posterior segment of the eye. Since the oxidative stress is a major factor in ocular inflammatory diseases, antioxidants or reactive oxygen scavengers are potent alternatives for this therapeutic application, and they may be useful to reduce the oxidative stress involved in the formation of senile cataract, which is the most common age-related eye complication. Moreover, antioxidants can protect against damage of retinal epithelia related to AMD, although in this case administration to the posterior segment of the eye should be used.

To date, three antioxidants have been considered for ocular therapy through LN: baicalin [92], quercetin [93], and epigallocatechin gallate [71].

Quercetin-loaded SLN showed good biocompatibility with corneal cells and enhanced permeation through cornea in excised porcine eyes [93]. In an in-depth study with baicalin loaded SLN, besides Draize test and ex vivo permeation studies on excised rabbit corneas, a pharmacokinetic analysis was performed through microdialysis of the anterior chamber of rabbits. SLN formulation promoted the prolonged release of baicalin and greatly increased the concentration of this drug in the ocular tissues when compared with the conventional eye drops [92].

Epigallocathechin gallate [71] was loaded in cationic LN which provided prolonged *ex vivo* transcorneal release; moreover, they proved to be safe and non-irritant. The use of drug-loaded cationic LN proved a promising strategy for the treatment of ocular diseases related to anti-oxidant and anti-inflammatory pathways.

8.1.4 Corneal wound healing

Calendula officinalis extract was entrapped in SLN for ocular delivery and demonstrated its wound-healing capacity on cultured Wong–Kilbourne derivative of Chang Conjunctival Epithelial Cells [94].

8.1.5 Immune-mediated ocular surface symptoms

Topical CsA has been successfully used in a variety of immune-mediated ocular surface phenomena like vernal conjunctivitis, dry eye syndrome and the prevention of corneal allograft rejection. However, CsA cannot be prepared in formulations based on the commonly used aqueous ophthalmic vehicles because of both its hydrophobicity and its extremely low aqueous solubility. Numerous formulations have been developed to avoid repeated injections and to achieve controlled release of CsA or to enhance efficacy of topical administration [95]. Due to its physico-chemical characteristics, it is suitable to be entrapped in LN intended for topical delivery [68, 69, 73, 75, 76, 96]. LN can load CsA, due to its lipophilicity, and enhance its bioavailabity. On the one hand, LN

can be taken up by the cornea epithelial cells, and on the other, LN surface can be modified (cationic lipids, chitosan, thiolated) to increase mucoadhesion.

8.1.6 Glaucoma

Since anti-glaucoma drugs are very potent, non-productive absorption from eye drops may cause serious side effects, i.e for β-antagonists on heart and airways in susceptible individuals: consequently, the development of an alternative to classic eye drops able to provide sustained and controlled drug delivery is a major challenge.

According to the literature, four drugs have been encapsulated in SLN for glaucoma treatment: pilocarpine [97], a cholinergic; timolol [98], a β-antagonist, which showed high and sustained permeation in human cornea construct; brimonidine, an α-agonist [99]; and methozolamide [74], a carbonic anhydrase inhibitor. Methozolamide was loaded in chitosan-coated SLN. *In vitro* (excised rabbit cornea, corneal hydration) and *in vivo* (IOP measurement and Draize test) results suggest that chitosan-coated SLN have a great potential for topical delivery, with the added advantage of being patient-friendly [74]. Brimonidine-loaded SLN and NLC were physically stable after autoclaving and showed excellent ocular tolerance, and no sign of ocular irritancy was observed in any of the rabbits' eyes during the study period [99].

Recently, melatonin receptors, a class of G protein-coupled receptors (GPCRs), have been detected in ciliary body, and melatonin and its analogues proved able to reduce the IOP in several species [41]. Melatonin has been loaded in cationic SLN as an innovative drug for glaucoma treatment. Melatonin-loaded SLN elicited a significant ($p < 0.01$) IOP reduction in rabbit eye (maximum IOP reduction: 7 mmHg), and their effect lasted approximately 24 h [70].

8.2 Delivery to the posterior segment of the eye

Despite the apparent advantages of LN as a drug delivery to the eye, the number of publications is even smaller in cases where these systems have been applied to deliver drugs to the posterior

segment of the eye. Intravitreal injection of ophthalmic suspensions containing triamcinolone acetonide has become increasingly popular to treat a broad spectrum of retinal diseases [100], despite the risks associated with this administration route. In this regard, Araujo et al. [91, 101] developed a formulation based on NLC containing triamcinolone acetonide for ocular instillation. NLC were detected in the retina, reaching a peak 40 min after administration, decreasing thereafter, and almost disappearing 160 min after administration.

However, some significant studies in the literature have aimed to exploit the potential of SLN as non viral vectors for ocular gene therapy, especially for retinal targeting [102, 103, 104, 105].

According to the European Medicine Agency [106], a gene therapy medicinal product generally consists of a vector or delivery formulation/system containing a genetic construct engineered to express a specific therapeutic sequence or protein responsible for the regulation, repair, addition or deletion of a genetic sequence.

The potential of gene therapy in the clinical management of retinal diseases has been demonstrated in four clinical trials [107, 108, 109, 110] with patients suffering from LCA treated with a viral vector. However, the limitations of viral gene therapy due to risk of immunogenicity and oncogenicity [111] or presence of virus particles in the brain after intravitreal injection [112] make the development of an efficacious non-viral vector for the eye of supreme importance [113]. In this sense, SLN have shown promising results in the treatment of XLRS.

SLN are complexed with the peptide protamine, which condenses and protects the genetic material and improves gene transduction, and a polysaccharide [114, 115]. The particles containing dextran as polysaccharide were administrated to Wistar rat eyes bearing a plasmid that encodes the enhanced green fluorescent protein (EGFP). After intravitreal injection, protein expression was mainly detected in ganglion cells, after subretinal administration in RPE and photoreceptors, and after topical administration preferably in corneal cells [103]. In a further study, when dextran was exchanged by hyaluronic acid a 7-fold increase in the efficacy was detected in vitro in ARPE-19 cells [104]. In another recent experimental work, the same SLN, complexed with protamine and

hyaluronic acid or dextran, were loaded with a plasmid encoding for both the EGFP and the therapeutic retinoschisin. Two weeks after injection to retinoshisin-deficient mice, as model of the human disease, EGFP and retinoschisin expression was detected in almost all retinal layers, which was maintained for at least two months after administration, and was related to a partial recovery of the retina. This work shows for the first time a successful gene transfer to retinoschisin-deficient animals using non-viral nanocarriers, with promising results that point to non-viral gene therapy as a feasible future therapeutic tool for retinal disorders [105].

More recently, the non-viral vectors based on SLN have been used for cell-specific gene delivery *in vivo* by using the cell-specific promoter mOPS. This promoter ensures specific expression of retinoschisin in photoreceptors, cells where retinoschisin is naturally produced. Using the mOPS as promoter of the gene that encodes the retinoschisin, two weeks after the injection of the vectors to retinoschisin-deficient mice, the improvement of the retina was slightly higher than that obtained with an ubiquitous promoter [116].

Despite these encouraging results obtained *in vitro* and *in vivo* for gene therapy in the retina, LN toxicity on retinal cells should be validated before applying to real patients, given the complex and sensitive physiology of the retina. In retinopathy, most studies on nanotoxicology have been performed as a part of studies on the therapeutic effect of nanoparticles, and reports focusing solely on the toxic effect of nanoparticles are few. Lipids are biocompatible components, but size, surface charge, concentration, and time of exposure are important factors in retinal toxicity [44].

9 Regulatory aspects and clinical trials

Since 2002, a number of ophthalmic lipid-based formulations have appeared on the market, and several more products are currently in the pipeline in preclinical and clinical trials [12]. The use of GRAS components, the large-scalable production methods, and the improved drug safety demonstrated by the use of lipid-based nanocarriers make these nanoformulations an ideal drug delivery system that fulfils the requirements for the pharmaceutical market [117; 118].

However, despite the potential therapeutic promise demonstrated by nanotechnology for ocular drug delivery, the bench to bed transition from patent inventions to marketed drug products has been insignificant. To date, the majority of recent clinical trials documented by the literature concern liposomal and polymeric nanoparticulate formulations: liposome-based ocular topical therapy has been studied in 72 patients affected by seasonal allergic rhinoconjunctivitis [119] and in 73 patients for the symptomatic treatment of dry eye, with encouraging results [120]; liposomally-entrapped ganciclovir was used for intravitreal injections in 5 AIDS patients suffering cytomegalovirus, increasing the time period required for reinjections [121]; an increased trans-scleral transport of carboplatin-loaded polymethylmethacrylate nanoparticles, without any associated short-term side effects was documented after posterior subtenon injection in 6 patients affected by advanced retinoblastoma [122].

On the contrary, most of the LN technologies discussed, instead, are still in the development and testing phase for commercial viability. Moreover, studies are in progress to assess ocular tolerance and nanotoxicity for prolonged use of nanoparticles [60].

10 Expert opinion

Considering that the ocular bioavailability of topically applied drugs is very poor, effective drug delivery to both the anterior and, especially, posterior segment of the eye is a challenging proposition for the pharmaceutical scientist. The main drawbacks are the elimination from lachrymal fluid and the corneal barrier after topical administration; moreover, for retinal targeting, alternative routes of administration (periocular and intravitreal) are generally needed, because BRB is the major obstacle to systemic drug delivery. In particular, repeated intravitreal administrations are associated with recurrent endophtalmitis, with sight-threatening effects.

Nanotechnology approaches can improve the therapeutic efficiency, compliance and safety of ocular drugs; and lipid-based nanocarriers are among the most biocompatible and versatile ones.

LN are among the most innovative colloidal systems used for drug delivery; they are very versatile and safe, being composed of biocompatible GRAS lipid molecules and produced through solvent free methods.

Mucoadhesion with consequent increase of pre-corneal retention time, and enhanced permeation due to cellular uptake by corneal epithelial cells, are the essential goals for topical LN delivery to the anterior segment of the eye. The small-sized biocompatible LN can be considered valid means to these ends both for their size-dependent ability to be endocytosed and for the retention-promoting effect of some of the surfactants used in the formulations. Moreover, functionalized, cationic or thermo-gelled formulations may also be used for this purpose. In fact, most experimental studies with LN concern topical administration covering a wide range of diseases that require a pharmacological treatment.

Because of their great kinetic stability and controlled release properties, LN can also be used as drug delivery systems to the posterior segment of the eye, thus reducing frequency of administration.

To date, various studies have been conducted on the use of LN in ocular drug delivery, although the different experimental models employed show that they are at different stages of development. An important limitation is the current lack of clinical trials: this can be attributed to a relatively recent approach compared to more established formulations (i.e. liposomes).

In fact, obtaining marketed LN formulations should be considered the ultimate goal for ocular drug delivery. However, the growing literature and patenting activity concerning LN for ocular delivery suggest that their full potential will be exploited in the next few years, considering that their versatility of production and ease of functionalization allow the entrapment of many active molecules. In this regard, it should be noted that delivery of macromolecules (especially peptides) within LN is a widely discussed research field, but no specific application for ocular delivery has yet been studied.

Moreover, within this field special attention should be paid to ocular genetic diseases, which currently cannot be treated pharmacologically. Gene delivery to the retina, obtained through intravitreal administration of plasmid complexed LN, has shown very promising potential. However, despite the encouraging results obtained and the safety of the vehicle, because of the sensitivity of the retina, this approach should be further validated before it is applied to real patients.

Disclosure statement

This work has been supported by the Basque Government's Department of Education, Universities and Investigation (IT-341-10) and by the Spanish Ministry of Economy and Competitiveness (SAF2014-53092-R). The authors would also thank Ricerca Locale 2014 (Italian MIUR) for funding.

The co-authors of this manuscript declare that no financial, commercial or other relationships of a declarable nature, relevant to the manuscript being submitted, is present.

Thanks are due to Mr. Adrian Belton for kindly revising the English version of the manuscript.

References

1. Sah AK, Suresh PK Recent advances in ocular drug delivery, with special emphasis on lipid based nanocarriers. Recent Pat Nanotechnol 2015; 9(2): 94-105.

2. Pignatello R, Carbone C, Puglia C et al. Ophthalmic applications of lipid-based drug nanocarriers: An update of research and patenting activity. Ther Deliv 2015; 6(11): 1297-1318.

3. Puglia C, Offerta A, Carbone C et al. Lipid nanocarriers (LNC) and their applications in ocular drug delivery. Curr Med Chem 2015; 22(13): 1589-1602.

4. Malhotra A, Minja FJ, Crum A et al. Ocular Anatomy and Cross-Sectional Imaging of the Eye. Semin Ultrasound CT MRI 2011; 32 (1): 2–13.

5. Patil BB and Dowd TC. Physiological Functions of the Eye. Curr Anaesth Crit Care 2000; 11 (6): 293–298.

6. Presland A. Applied Ocular Physiology and Anatomy. Anaesth Intens Care Med 2007; 8 (9): 379–382.

7. Presland A and Price J. Ocular Anatomy and Physiology Relevant to Anaesthesia. Anaesth Intens Care Med 2014; 15 (1): 20–25.

8. Wichmann, W and Müller-Forell W. Anatomy of the Visual System. Eur J Radiol 2004; 49 (1): 8–30.

9. Hodges RR, Dartt DA. Tear Film Mucins: Front Line Defenders of the Ocular Surface; Comparison with Airway and Gastrointestinal Tract Mucins. Exp Eye Res 2013; 117: 62–78.

10. Bron AJ, Tiffany JM, Gouveia SM et al. Functional aspects of the tear film lipid layer. Exp Eye Res 2004; 78: 347–360.

11. Dartt DA. Interaction of EGF family growth factors and neurotransmitters in regulating lacrimal gland secretion. Exp Eye Res 2004; 78: 337–345.

12. Gan L, Wang J, Jiang M, et al. Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. Drug Discov Today 2013; 18: 290-297

•=of importance: it shows marketed lipid based nano-formulations

13. Goel M, Picciani RG, Lee RK et al. Aqueous humor dynamics: a review. Open Ophthalmol J 2010; 3(4): 52-59.

14. Meek KM. The Cornea and Sclera. In: Collagen: Structure and Mechanics. P. Fratzl (ed.) Springer Science+Business Media, LLC 2008

15. Presland A, and Myatt J. Ocular anatomy and physiology relevant to anaesthesia. Anaesth Intens Care Med 2010; 11: 438-443

16. Murthy KR, Goel R, Subbannayya Y, et al. Proteomic analysis of human vitreous humor. Clin Proteomics. 2014; 11(1): 29.

17. Souto EB, Doktorovova S, Gonzalez-Mira E et al. Feasibility of Lipid Nanoparticles for Ocular Delivery of Anti-Inflammatory Drugs. Curr Eye Res 2010; 35(7): 537–552.

18. Cunha-Vaz J, Bernardes R and Lobo C. Blood-retinal barrier. Eur J Ophthalmol 2011; 21: S3-S9

19. Campbell M and Humphries P. The blood-retina barrier: tight junctions and barrier modulation. Adv Exp Med Biol 2012; 763: 70-84

20. Eljarrat-Binstock E, Pe'er J, and Domb AJ. New techniques for drug delivery to the posterior eye segment. Pharm Res 2010; 27: 530–543.

21. del Amo EM., and Urtti A. Current and future ophthalmic drug delivery systems. A shift to the posterior segment. Drug Discov Today. 2008; 13: 135–143.

22. Mannermaa E. In Vitro Model of Retinal Pigment Epithelium for Use in Drug Delivery Studies. Kuopio: University of Eastern Finland; 2010.

23. Duvvuri S, Majumdar S, and Mitra A.K. Drug delivery to the retina: challenges and opportunities. Expert Opin Biol Ther. 2003; 3: 45–56.

24. Hsu J. Drug delivery methods for posterior segment disease. Curr Opin Ophthalmol 2007; 18: 235–239.

25. Wadhwa S, Paliwal R, Paliwal SR et al. Nanocarriers in Ocular Drug Delivery: An Update Review. Curr Pharm Des 2009; 15: 2724-2750.

26. Sahay G, Alakhova DY, Kabanov AV. Endocytosis of nanomedicines. J Control Release 2010; 145(3): 182-195.

27. Bucolo C, Drago F, and Salomone S. Ocular Drug Delivery: A Clue from Nanotechnology. Front Pharmacol 2012; 3: 2002–2004.

28. Thrimawithana TR, Young S, Bunt CR et al. Drug delivery to the posterior segment of the eye. Drug Discov Today 2011; 16: 271-277.

29. Bäckström G, Lundberg B, Behndig A. Intracameral acetylcholine effectively contracts pupils after dilatation with intracameral mydriatics. Acta Ophthalmol 2013; 91(2):123-126.

30. Wadhwa S, Paliwal R, Paliwal SR, et al. Nanocarriers in ocular drug delivery: an update review. Curr Pharm Des 2009; 15: 2724–2750.

31. Ausayakhun S, Yuvaves P, Ngamtiphakom S, et al. Treatment of cytomegalovirus retinitis in AIDS patients with intravitreal ganciclovir. J Med Assoc Thai 2005; 88: S15–S20.

32. Mikhail M, Sallam A. Novel Intraocular Therapy in Non-infectious Uveitis of the Posterior Segment of the Eye. Med Hypothesis Discov Innov Ophthalmol 2013; 2(4): 113–120.

33. Solinís MÁ, del Pozo-Rodríguez A, Apaolaza PS, et al. Treatment of ocular disorders by gene therapy. Eur J Pharm Biopharm 2015; 95 (Pt B): 331-342.

34. Bloquel C, Bourges JL, Touchard E, et al. Non-viral ocular gene therapy: potential ocular therapeutic avenues. Adv Drug Deliver Rev 2006; 58: 1224–1242.

35. Bucolo C, and Drago F. Carbon Monoxide and the Eye: Implications for Glaucoma Therapy. Pharmacol Ther 2011; 130 (2): 191–201.

36. Zhou HY, Hao JL, Wang S et al. Nanoparticles in the ocular drug delivery. Int J Ophthalmol 2013; 6(3): 390-396.

37. Elbadawy HM, Gailledrat M, Desseaux C, et al. Targeting herpetic keratitis by gene therapy. J Ophthalmol 2012; 2012:594869.

38. Cho YK, Uehara H, Young JR et al. Flt23k nanoparticles offer additive benefit in graft survival and anti-angiogenic effects when combined with triamcinolone. Invest Ophthalmol Vis Sci 2012; 53(4): 2328-2336

39. Hattori M, Shimizu K, Katsumura K, et al. Effects of all-trans retinoic acid nanoparticles on corneal epithelial wound healing. Graefes Arch Clin Exp Ophthalmol 2012; 250(4): 557-563

40. Kim NJ, Harris A, Gerber A et al. Nanotechnology and glaucoma: a review of the potential implications of glaucoma nanomedicine. Br J Ophthalmol 2014; 98: 427–431.

41. Bucolo C, Platania CB, Reibaldi M, et al. Controversies in Glaucoma: Current Medical Treatment and Drug Development. Curr Pharm Des 2015; 21(32): 4673-81.

42. Bucolo C, Salomone S, Drago F, et al. Pharmacological Management of Ocular Hypertension: Current Approaches and Future Prospective. Curr Opin Pharmacol 2013; 13 (1): 50–55.

43. Musumeci T, Bucolo C, Carbone C, et al. Polymeric Nanoparticles Augment the Ocular Hypotensive Effect of Melatonin in Rabbits. Int J Pharm 2013; 440 (2): 135–140.

44. Dong Hyun Jo, Tae Geol Lee and Jeong Hun Kim. Nanotechnology and Nanotoxicology in Retinopathy. Int J Mol Sci 2011; 12: 8288-8301.

45. Preeti K, Suresh M and Abhishek K. Nanocarriers for ocular delivery for possible benefits in the treatment of anterior uveitis: focus on current paradigms and future directions. Expert Opin Drug Deliv 2014; 11(11): 1747-1768

46. Renfro L, Snow JS. Ocular effects of topical and systemic steroids. Dermatol Clin. 1992;10: 505–512.

47. Schalnus R. Topical nonsteroidal anti-inflammatory therapy in ophthalmology. Ophthalmologica. 2003; 217: 89–98.

48. Sneha Solanki, Manisha Rathi, Sumeet Khanduja et al. Recent trends: Medical management of infectious keratitis. Oman J Ophthalmol 2015; 8(2): 83–85.

49. Keynan Y, Finkelman Y, Lagacé-Wiens P. The microbiology of endophthalmitis: global trends and a local perspective. Eur J Clin Microbiol Infect Dis 2012; 31: 2879–2886.

50. Camber O. Studies on corneal permeability and an evaluation of prostaglandin F(2α) pro-drugs and sodium hyaluronate. Acta Pharm Suec 1988; 25(3): 181.

51. Surajit D. Corneal cell culture models: a tool to study corneal drug absorption. Expert Opin Drug Metab Toxicol 2011; 7(5): 529-532.

52. Sarmento B, Andrade F, Baptista da Silva S et al. Cell-based in vitro models for predicting drug permeability. Expert Opin Drug Metab Toxicol 2012; 8(5): 607-621.

53. Vinardell MP, Mitjans M. Alternative Methods for Eye and Skin Irritation Tests: An Overview. J Pharm Sci 2008; 97: 46–59.

54. Marcondes PF, Rodrigues EB, Maia M. Retinal and Ocular Toxicity in Ocular Application of Drugs and Chemicals – Part I: Animal Models and Toxicity Assays. Ophthalmic Res 2010; 44: 82–104

55. Grossniklaus HE, Kang SJ, Berglin L. Animal models of choroidal and retinal neovascularization. Prog Retin Eye Res 2010; 29: 500-519

56. Weber BH., et al. Inactivation of the murine X-linked juvenile retinoschisis gene, Rs1h, suggests a role of retinoschisin in retinal cell layer organization and synaptic structure. Proc Natl Acad Sci USA 2002; 99: 6222–6227.

57. Veleri S, Lazar CH, Chang B, et al. Biology and therapy of inherited retinal degenerative disease: insights from mouse models. Dis Model Mech 2015; 8:109–129.

58. Mowat FM, Breuwer AR, Bartoe JT, et al. RPE65 gene therapy slows cone loss in Rpe65-deficient dogs. Gene Ther. 2013; 20(5): 545-55.

59. Rivas MA, Vecino E. Animal models and different therapies for treatment of retinitis pigmentosa. Histol Histopathol. 2009; 24(10): 1295-322.

60. Ako-Adounvo AM, Nagarwal RC, Oliveira L, et al. Recent patents on ophthalmic nanoformulations and therapeutic implications. Recent Pat Drug Deliv Formul 2014; 8: 193-201.

61. Almeida H, Amaral MH, Lobão P at al. Applications of Polymeric and Lipid Nanoparticles in Ophthalmic Pharmaceutical Formulations: Present and Future Considerations. J Pharm Pharm Sci 2014; 17(3): 278-293.

•=of importance: it compares lipid and polymeric nanoparticles for ocular delivery

62. Kothuri MK, Pinnamaneni S, Das NG, et al. Microparticles and nanoparticles in ocular drug delivery. In: Mitra, A.K., ed. Ophthalmic Drug Delivery Systems. NY: Informa Healthcare; 2003; pp. 437–466.

63. Battaglia L and Gallarate M. Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery. Expert Opin Drug Deliv 2012; 9(5): 497-508

••= of considerable importance: it describes new formulative approaches and treends in SLN field

64. Seyfoddin A, Shaw J, and Al-Kassas R. Solid lipid nanoparticles for ocular drug delivery. Drug Deliv. 2010; 17: 467–489.

65. Mehnert W, and Mäder K. Solid lipid nanoparticles: Production, characterization and applications. Adv Drug Deliv Rev 2001; 47:165–196.

66. Müller RH, and Olbrich C. Lipid matrix-drug conjugates particle for controlled release of active ingredient. US6770299; 2004.

67. Xiang Li, Shu-fang Nie, Jun Kong et al. A controlled-release ocular delivery system for ibuprofen based on nanostructured lipid carriers Int J Pharm 2008; 363: 177–182.

68. Başaran E, Demirel M, Sırmagül B et al. Cyclosporine-A incorporated cationic solid lipid nanoparticles for ocular delivery. J Microencapsul 2010; 27(1): 37–47.

69. Niu M, Shi K, Sun Y et al. Preparation of CyA-loaded solid lipid nanoparticles and application on ocular preparations. J Drug Del Sci Tech 2008; 18(4): 293-297.

70. Leonardi A, Bucolo C, Drago F, et al. Cationic Solid Lipid Nanoparticles Enhance Ocular Hypotensive Effect of Melatonin in Rabbit. Int J Pharm 2015; 478 (1): 180–186.

71. Fangueiro JF, Calpena AC, Clares B, et al. Biopharmaceutical Evaluation of Epigallocatechin Gallate-Loaded Cationic Lipid Nanoparticles (EGCG-LNs): In Vivo, in Vitro and Ex Vivo Studies. Int J Pharm 2016; 502 (1-2): 161–169.

72. Qiuhua L, Junming Z, Xiangrong Z. Nanostructured lipid carrier (NLC) coated with Chitosan Oligosaccharides and its potential use in ocular drug delivery system. Int J Pharm 2011; 403: 185–191.

73. Battaglia L, D'Addino I, Peira E et al. Solid lipid nanoparticles prepared by coacervation method as vehicles for ocular cyclosporine. J Drug Del Sci Tech 2012; 22(2): 125-130.

74. Wang F, Chen L, Zhang D et al. Methazolamide-loaded solid lipid nanoparticles modified with low-molecular weight chitosan for the treatment of glaucoma: vitro and vivo study. J Drug Target 2014; 22(9): 849–858.

75. Shen J, Deng Y, Jin X et al. Thiolated nanostructured lipid carriers as a potential ocular drug delivery system for cyclosporine A: Improving in vivo ocular distribution. Int J Pharm 2010; 402: 248–253.

76. Shen J, Wang Y, Ping Q et al. Mucoadhesive effect of thiolated PEG stearate and its modified NLC for ocular drug delivery. J Control Release 2009; 137: 217–223.

77. Hao J, Wang X, Bi Y et al. Fabrication of a composite system combining solid lipid nanoparticles and thermosensitive hydrogel for challenging ophthalmic drug delivery. Colloids Surf B Biointerfaces 2014; 114: 111– 120.

78. Leonardi A, Bucolo C, Romano GL et al. Influence of different surfactants on the technological properties and in vivo ocular tolerability of lipid nanoparticles. Intl J Pharm 2014; 470: 133–140.

79. Cavalli R, Gasco MR, Chetoni P et al. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. Int J Pharm 2002; 238: 241–245.

80. JHao J, Fang X, Zhou Y et al. Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box-Behnken design. Int J Nanomedicine 2011; 6: 683–692.

81. Kalam MA, Sultana Y, Ali A, et al. Part I: Development and optimization of solid-lipid nanoparticles using Box-Behnken statistical design for ocular delivery of gatifloxacin. J Biomed Mater Res A 2013; 101A(6): 1813-1827.

82. Kalam MA, Sultana Y, Ali A et al. Part II: Enhancement of transcorneal delivery of gatifloxacin by solid lipid nanoparticles in comparison to commercial aqueous eye drops. J Biomed Mater Res A 2013; 101A(6): 1828-1836.

83. Kalam MA, Sultana Y, Ali A et al. Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system. J Drug Targ 2010; 18(3): 191–204.

84. Salman M, Ahad A, Aslam M, et al. Application of Box–Behnken design for preparation of levofloxacin-loaded stearic acid solid lipid nanoparticles for ocular delivery : optimization, in vitro release, ocular tolerance, and antibacterial activity. Int J Biol Macromolec 2016; 85: 258–270.

85. Mohanty B, Majumdar D, Mishra S et al. Development and characterization of itraconazole loaded solid lipid nanoparticles for ocular delivery. Pharm Dev Technol 2015; 20(4): 458–464.

86. Kakkar S, Karuppayil SM, Raut JS, et al. Lipid-Polyethylene Glycol Based Nano-Ocular Formulation of Ketoconazole. Int J Pharm 2015; 495 (1): 276–289.

87. Seyfoddin A and Al-Kassas R. Development of solid lipid nanoparticles and nanostructured lipid carriers for improving ocular delivery of acyclovir. Drug Dev Ind Pharm 2013; 39(4): 508–519.

88. Attama AA, Reichl S, Muller-Goymann CC. Diclofenac sodium delivery to the eye: In vitro evaluation of novel solid lipid nanoparticle formulation using human cornea construct. Int J Pharm 2008; 355: 307–313.

89. Gonzalez-Mira E, Egea MA, Garcia ML et al. Design and ocular tolerance of flurbiprofen loaded ultrasound-engineered NLC. Colloids Surf B Biointerfaces 2010; 81: 412–421.

90. Hippalgaonkar K, Adelli GR, Hippalgaonkar K. et al. Indomethacin-loaded solid lipid nanoparticles for ocular delivery: Development, characterization, and in vitro evaluation. J Ocul Pharmacol Ther 2013; 29(2): 216-228.

91. Araújo J, Gonzalez-Mira E, Egea MA et al. Optimization and physicochemical characterization of a triamcinolone acetonide-loaded NLC for ocular antiangiogenic applications. Int J Pharm 2010; 393: 167–175.

92. Zhidong Liu, Xinhua Zhang, Haoyun Wu et al. Preparation and evaluation of solid lipid nanoparticles of baicalin for ocular drug delivery system in vitro and in vivo. Drug Dev Ind Pharm 2011; 37(4): 475–481.

93. Chi-Hsien Liu, Yun-Chun Huang, Jhe-Wei Jhang et al. Quercetin delivery to porcine cornea and sclera by solid lipid nanoparticles and nanoemulsion. RSC Adv 2015; 5: 100923–100933.

94. Arana L, Salado C, Vega S et al. Solid lipid nanoparticles for delivery of Calendula officinalis extract. Colloids Surf B Biointerfaces 2015; 135: 18–26.

95. Lallemand F, Felt-Baeyens O, Besseghir K et al. Cyclosporine A delivery to the eye: A pharmaceutical challenge. Eur J Pharm Biopharm 2003; 56: 307–318.

96. Gokce EH, Sandri G, Bonferoni MC et al. Cyclosporine A loaded SLNs: Evaluation of cellular uptake and corneal cytotoxicity. Int J Pharm 2008; 364: 76–86.

97. Cavalli R, Morel S, Gasco MR et al. Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion pair. Int J Pharm 1995;117: 243-246.

98. Attama AA, Reichl S and Müller-Goymann CC. Sustained Release and Permeation of Timolol from Surface-Modified Solid Lipid Nanoparticles through Bioengineered Human Cornea. Curr Eye Res 2009; 34: 698–705.

99. El-Salamouni NS, Farid RM, El-Kamel AH, et al. Effect of sterilization on the physical stability of brimonidine-loaded solid lipid nanoparticles and nanostructured lipid carriers. Int J Pharm 2015; 496 (2): 976–983.

100. Jonas J.B. Intravitreal triamcinolone acetonide: a change in a paradigm. Ophthalmic Res 2006; 38:218–245.

101. Araujo J, Nikoli S, Egea MA, et al. Nanostructured lipid carriers for triamcinolone acetonide delivery to the posterior segment of the eye. Colloids Surf B Biointerfaces 2011; 88: 150–157.

102. del Pozo-Rodríguez A, Delgado D, Gascón AR et al. Lipid nanoparticles as drug/gene delivery systems to the retina. J Ocul Pharmacol Ther 2013; 29(2): 173-188.

••= of considerable importance: it describes innovative application of SLN to gene delivery to retina

103. Delgado D, Del Pozo-Rodríguez A, Solinís MA et al. Dextran and protamine-based solid lipid nanoparticles as potential vectors for the treatment of X-linked juvenile retinoschisis. Hum Gene Ther 2011; 23(4): 345-355.

104. Apaolaza PS, Delgado D, Del Pozo-Rodríguez A et al. A novel gene therapy vector based on hyaluronic acid and solid lipid nanoparticles for ocular diseases. Int J Pharm 2014; 465: 413–426.

105. Apaolaza PS, Del Pozo-Rodríguez A, Torrecilla J et al. Solid lipid nanoparticle-based vectors intended for the treatment of X-linked juvenile retinoschisis by gene therapy: In vivo approaches in Rs1h-deficient mouse model. J Control Release 2015; 217: 273–283.

106. EMA. Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products. Draft. EMA/CAT/80183/2014. European Medicines Agency, 2015.

107. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med 2008; 358: 2231–2239.

108. Banin E, Bandah-Rozenfeld D, Obolensky A, et al. Molecular anthropology meets genetic medicine to treat blindness in the North African Jewish population: human gene therapy initiated in Israel. Hum Gene Ther 2010; 21: 1749–1757.

109. Cideciyan AV, Aleman TS, Boye SL, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. Proc Natl Acad Sci USA 2008; 105: 15112–15117.

110. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 2008; 358: 2240–2248.

111. Kumar-Singh, R. Barriers for retinal gene therapy: separating fact from fiction. Vis Res 2008; 48: 1671–1680.

112. Provost N, Le Meur G, Weber M, et al. Biodistribution of rAAV vectors following intraocular administration: evidence for the presence and persistence of vector DNA in the optic nerve and in the brain. Mol Ther 2005; 11: 275–283.

113. Cai X, Conley S, Naash M. Nanoparticle applications in ocular gene therapy. Vision Res 2008; 48: 319–324

114. Rodríguez-Gascón A, Solinís MA, del Pozo-Rodríguez A, et al. Lipid nanoparticles for gene therapy. US 20120183589 A1, 2012.

115. Rodríguez-Gascón A, Solinís MA, del Pozo-Rodríguez A, et al. Lipid nanoparticles for treating ocular diseases. WO 2012085318 A1, 2012.

116. Apaolaza, A. del Pozo-Rodríguez, M.A. Solinís et al. Structural recovery of the retina in a retinoschisin-deficient mouse after gene replacement therapy by solid lipid nanoparticles. Biomaterials 2016; 90: 40-49

117. Lim SB, Banerjee A and Önyüksel H. Improvement of drug safety by the use of lipid-based nanocarriers. J Control Release 2012; 163:34-45

118. Beloqui A, Solinís MÁ, Rodríguez-Gascón A, et al. Nanostructured Lipid Carriers: promising drug delivery systems for future clinics. Nanomed 2016; 12: 143-161]

119. Böhm M, Avgitidou G, El Hassan E, et al. Liposomes: a new non-pharmacological therapy concept for seasonal-allergic-rhinoconjunctivitis. Eur Arch Otorhinolaryngol 2012; 269: 495–502

120. Hofauer B, Bas M, Manour N, et al. Effekt liposomaler Lokaltherapie auf die Sicca-Symptomatik des primären Sjögren-Syndroms. HNO 2013; 61: 921–927

121. Díaz-Llopis M, Martos MJ, España E, et al. Liposomally entrapped ganciclovir for the treatment of cytomegalovirus retinitis in AIDS patients. Experimental toxicity and pharmacokinetics, and clinical trial. Doc Ophthalmol 1992; 82: 297–305

122. Kalita D, Shome D, Jain VG, et al. In vivo intraocular distribution and safety of periocular nanoparticle carboplatin for treatment of advanced retinoblastoma in humans. Am J Ophthalmol 2014; 157: 1109–1115

Table 1: Advantages and drawbacks of colloidal carriers in ocular delivery

	Advantages	Drawbacks	References
Colloidal carriers	- small particle size, promoting endocytosis - adhesive properties, with improvement of drug pre-ocular retention - PgP inhibition - improvement of bioavailability of poorly water-soluble drugs - protection of sensitive molecules (especially vs enzyme inactivation) - targeted and controlled release characteristics	- tissue accumulation and aggregation in the eye - low drug/matrix ratio: suitable only for potent drugs, with a low therapeutic dose	[12, 60, 61]
	Additional advantages	Specific drawbacks	
Polymeric nanoparticles (nanospheres, nanocapsules)	- biodegradability - biocompatibility - mucoadhesiveness - ease and low cost of production - possibility to freeze-dry and reconstitute, with increased long-term stability	- possible systemic toxic effects from polymer degradation products - possible toxicity from residual organic solvents	[61]
Nanoemulsions	- reconstruction of the tear film - improving the 'wettability' of tear film	- sticky feel and subsequent intolerance because of surfactants	[12, 60]
Liposomes	- made of natural biocompatible phospholipids - encapsulation of both hydrophilic (in the inner acqueous core) and hydrophobic (in the vesicle membrane) molecules - flexibility, allowing repeated intraocular injections	- lower drug loading capacity compared to nanoparticles	[60, 62]
Niosomes	- biodegradability - biocompatiblity - non-immunogenicity - encapsulation both hydrophilic and hydrophobic drug molecules	- lower drug loading capacity compared to nanoparticles	[12]
LN (SLN, NLC, LDC)	- low or absence of in vivo toxicity, related to the use of GRAS excipients - good long-term stability - economic and solvent free production techniques - easy production at large scale - possibility to be autoclaved or sterilized - great kinetic stability compared to liposomes and niosomes	- drug expulsion during storage (for SLN)	[61, 64, 65]

Table 2: Case study of LN used for ocular drug/gene delivery

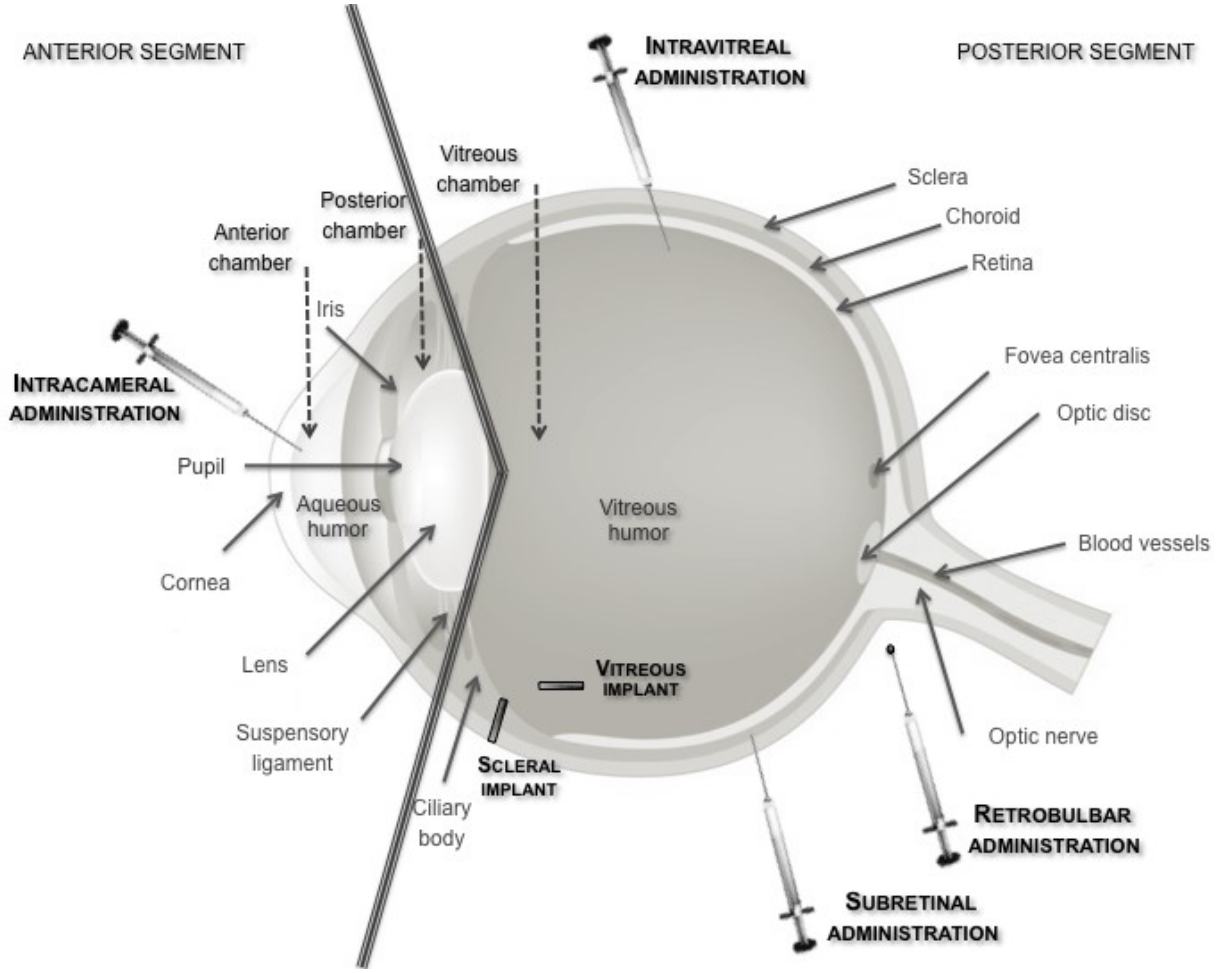
Therapeutic aim	Drug category	Addressed issues	Drug	Formulation	Preparation method	Administration route	Pharmacological tests performed	Toxicity test performed	references
Treatment of ocular infectious diseases	antibiotic	enhanced precorneal retention; sustained drug release; increased biocidal activity	tobramycin	SLN	warm microemulsion dilution	topical	pre-ocular retention on rabbits; ocular pharmacokinetic in rabbits	-	79
			chloramphenicol	SLN	melt-emulsion ultrasonication	topical	-	-	80
			gatifloxacine	Cationic SLN	warm microemulsion dilution	topical	*ex vivo* corneal permeation;	determination of corneal hydration levels	81, 82, 83
			levofloxacin	SLN	emulsion-solvent evaporation	topical	*ex vivo* corneal permeation	CAM test	84
	antifungal		itraconazole	SLN	melt-emulsion ultrasonication	topical	*ex vivo* corneal permeation	determination of corneal hydration levels	85
			ketoconazol	SLN	HPH		*ex vivo* corneal permeation	Draize test	86
	antiviral		acyclovir	SLN/NLC	modified warm microemulsion dilution	topical	*ex vivo* corneal permeation	determination of corneal hydration levels	87
Ocular inflammation treatment	NSAID	precorneal retention; corneal-conjunctival	diclofenac	SLN	HPH	topical	*in vitro* permeation on human corneal constructs	-	88

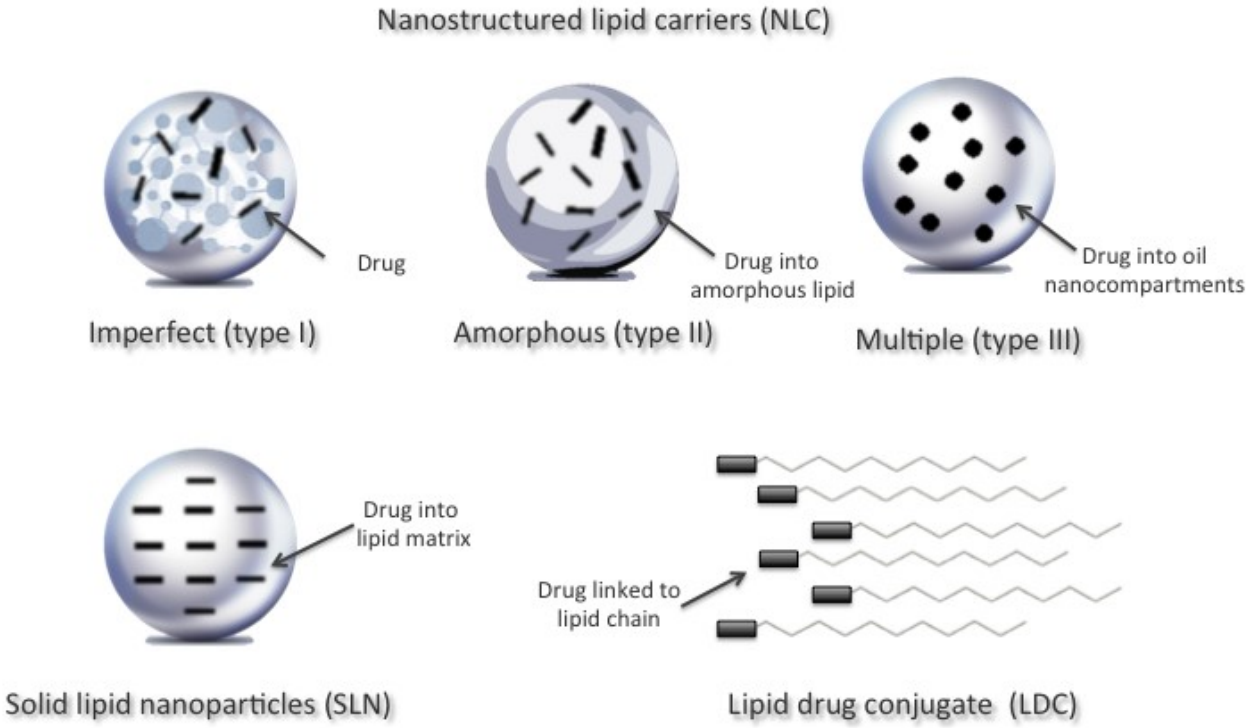
		uptake; enhancement; sustained drug release	ibuprofen	Cationic NLC	melt-emulsion ultrasonication	topical	pre-ocular retention on rabbits; *ex vivo* corneal permeation	Draize test; determination of corneal hydration levels	67
			flurbiprofen	NLC	melt-emulsion ultrasonication	topical	-	Draize test; EYTEX™	89
				Chitosan coated NLC	melt-emulsion ultrasonication	topical	pre-ocular retention on rabbits; *ex vivo* corneal permeation	-	72
			indomethacin	SLN	HPH	topical	-	-	90
	corticosteroid		triamcinolone acetonide	NLC	HPH	topical	-	-	91
	corticosteroid	controlled release	triamcinolone acetonide	NLC	HPH	intravitreal	*in vivo* permeation to the posterior segment of the eye	-	101
ocular inflammation treatment through oxidayive stress reduction	antioxidant	precorneal retention; corneal-conjunctival uptake enhancement; controlled drug release	baicalin	SLN	melt-emulsion ultrasonication	topical	ocular pharmacokinetic; *ex vivo* corneal permeation	Draize test; determination of corneal hydration levels	92
			quercetin	SLN	melt-emulsion ultrasonication	topical	*ex vivo* corneal permeation	-	93
			epigallocatechin gallate	Cationic SLN	Hot/cold multiple emulsions	topical	*ex vivo* corneal permeation test;	determination of corneal hydration levels;	71

								CAM test	
ocular wound healing	lenitive extract	solubility enhancement	*Calendula officinalis* extract	SLN	warm microemulsion dilution	topical	conjunctival cell cultures	-	94
Treatment of immune-mediated ocular surface symptoms (dry eye syndrome, preventing corneal rejection, ocular inflammation)	Immuno-suppressant	solubility enhancement; controlled release; precorneal retention	CsA	Thiolated NLC	melt-emulsification	topical	ocular pharmacokinetic in rabbits	-	75
				Cationic SLN	HPH	topical	Drug levels in aqueous and vitreous humor after topical administration in sheep	-	68
				Chitosan coated SLN	coacervation	topical	*ex vivo* corneal permeation;	BCOP	73
				SLN	melt-emulsion ultrasonication	topical	*in vitro* permeation on immortalized cells; ex vivo corneal permeation	-	96
				Cationic SLN	melt-emulsion ultrasonication	topical	pre-ocular retention on rabbits	-	69
				Thiolated NLC	melt-emulsification	topical	pre-ocular retention on rabbits;	Draize test	76
Glaucoma treatment	beta-antagonist	reduction of non-productive absorption; sustained drug release	timolol	SLN	melt-emulsification	topical	*in vitro* permeation on human cornea construct	-	98
	carbonic anhydrase inhibitor		methozolamide	Chitosan coated SLN	emulsion-solvent evaporation	topical	*ex vivo* corneal permeation; in vivo IOP measurement in rabbits	Draize test; determination of corneal hydration	74

								levels	
	cholinergic		pilocarpine	SLN	warm microemulsion dilution	topical	-	-	97
	IOP reducing agent		melatonin	Cationic SLN	Solvent injection	topical	*in vivo* IOP measurement in rabbits	Draize test	70
	α2 agonist		brimonidine	SLN and NLC	melt-emulsion	topical	-	Draize test	99-
gene delivery for XLRS	plasmid	Plasmid complexation, internalization and expression by retinal cells	EGFP plasmid; retinoschisin plasmid	SLN	emulsion-solvent evaporation	subretinal	ARPE-19 cell cultures; in vivo transfection studies	-	103,104,105, 116

SLN: solid lipid nanoparticles; NLC: nanostructured lipid carriers; HPH: high pressure homogenization; NSAID: Non steroidal anti-inflammatory drugs; BCOP: cornea opacity and permeability test; IOP: intraocular pressure; EGFP: enhanced green fluorescent protein; ARPE-19: human retinal pigment epithelial cells; XLRS: X-linked juvenile retinoschisis.


ANTERIOR SEGMENT
INTRAVITREAL ADMINISTRATION
POSTERIOR SEGMENT
Vitreous chamber
Posterior chamber
Anterior chamber
Iris
Sclera
Choroid
Retina
INTRACAMERAL ADMINISTRATION
Fovea centralis
Optic disc
Pupil
Aqueous humor
Vitreous humor
Blood vessels
Cornea
Lens
VITREOUS IMPLANT
Suspensory ligament
Optic nerve
SCLERAL IMPLANT
Ciliary body
RETROBULBAR ADMINISTRATION
SUBRETINAL ADMINISTRATION


Nanostructured lipid carriers (NLC)
Drug
Drug into amorphous lipid
Drug into oil nanocompartments
Imperfect (type I)
Amorphous (type II)
Multiple (type III)
Drug into lipid matrix
Drug linked to lipid chain
Solid lipid nanoparticles (SLN)
Lipid drug conjugate (LDC)