PHARMACEUTICAL MICRO- AND NANOSCALE APPROACHES FOR EFFICIENT TREATMENT OF OCULAR DISEASES

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ACKNOWLEDGMENTS- ISCIII-FEDER Red Temática de Investigación Cooperativa en Oftalmología RETICS (Oftared) RD12/0034/0003 and RD12/0034/0014, Complutense Research Group UCM 920415-GR3/14, Spanish Ministry of Economy and Competitiveness MICINN-MAT 2013–43127R and Spanish Ministry of Health and Consumption. Spanish Fund for Health Research FIS PI12/02285.

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ABSTRACT

Efficient treatment of ocular diseases can be achieved thanks to the proper use of ophthalmic formulations based on emerging pharmaceutical approaches. Among them, micro- and nanotechnology strategies result of great interest in the development of novel drug delivery systems to be used in the ocular therapy. The location of the target site in the eye as well as the ophthalmic disease will determine the route of administration (topical, intraocular, periocular and suprachoroidal administration) and the most adequate device. In this review, we discuss the use of colloidal pharmaceutical systems (nanoparticles, liposomes, niosomes, dendrimers and microemulsions); microparticles (microcapsules and microspheres) and hybrid systems (combination of different strategies) in the treatment of ophthalmic diseases. Emphasis has been placed in the therapeutic significance of each drug delivery device for clinical translation.

KEYWORDS: microparticles, microspheres, nanotechnology, nanoparticles, ocular, anterior segment, posterior segment.

INTRODUCTION

The eye is divided in anterior segment (from cornea to lens, bath by aqueous humor) and posterior segment (from lens to the eye fundus, filled by the vitreous) Fig 1. Ocular drug delivery is one of the most challenging fields of pharmaceutical research. As the eye has several defense mechanisms to prevent the entrance of exogenous substances, the drug must overcome different protecting barriers to achieve the target tissue. In this context, the active substance must overcome static barriers (cornea, sclera, and retina including blood aqueous and blood-retinal barriers) and/or dynamic barriers (choroidal and conjunctival blood flow, lymphatic clearance, and tear dilution) that are protecting the eye, to reach the target site.

Topical administration is the preferred route to reach the anterior segment of the eye or to treat diseases affecting the ocular surface. However, it has been estimated that only a low percentage (around 5%) of the administered drug is able to reach the aqueous humor or get access inside the eye when conventional eye drops are used. This poor ocular bioavailability is due to different events such as the short residence time of formulations on the ocular surface, to drainage of the solution, tear turnover, dilution by lacrimation and the low corneal permeability [1]. Several therapeutic approaches (mainly nanosystems) have been developed to increase ocular drug bioavailability. After topical administration, they are directed toward the increase of the retention time of drugs on the ocular surface and, in some cases, to enhance the corneal transport, reducing the number of applications per day and increasing the patients' compliance.

When the target tissue is located in the posterior segment of the eye, mainly one or more than one type of retinal neurons (Fig 2), the use of topical or systemic administrations is ineffective because of the above-mentioned ocular barriers. Subsequently, for the treatment of these pathologies, the intraocular administration is preferred. In the cases in which the drug is able to cross the different tissues to reach the back of the eye, a less invasive route (periocular injection)-can be also considered. The main lack-point of these administrations is the need of repeated injections to achieve therapeutic drug levels for prolonged periods of time, which is generally necessary to treat the chronic posterior segment pathologies. In that sense, intraocular and periocular implants and microparticulate delivery systems may address the risk of side effects associated with repeated injections, as they are able to release the drug for long periods of time. At this level, nanosystems are useful tools to protect the active substance from the external environment and/or to target the drug to the ocular tissues.

In the past decades, micro- and nanosystems (liposomes, nanoparticles, niosomes, microemulsions and more recently dendrimers) have emerged as interesting drug delivery alternatives in ophthalmology. However, ophthalmic pharmaceutical formulations must fulfill some restricted specifications different to those designed for other targets, such as sterility, compatibility and tolerability. This review is dedicated to describe the most promising research performed in this field. The information has been classified according to the ocular administration route selected (topical, intraocular and periocular). A novel approach currently under investigation is the use of suprachoroidal space located between the sclera and choroid as a reservoir for active substances (Fig 1).

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TOPICAL ADMINISTRATION

As cited previously, the administration of drugs on the ocular surface by topical instillation of eye drops is the most common drug delivery route for the treatment of ocular pathologies affecting the ocular surface or when the drug has to reach the anterior segment. However, having clear advantages such as an easy self-application, the main drawback of this route is the rapid clearance of the drug from the ocular surface and the reduced permeability of the ocular tissues. Once the drug reaches the ocular surface it has to be diluted in the precorneal film (Fig 3) and subsequently cross the cornea (Fig 4) to reach the aqueous humor. This fact reduces the bioavailability of drugs, limiting the use of the topical route to the treatment of anterior segment diseases.

Several technological strategies to increase the bioavailability of drugs by topical administration are under study. These strategies are based on the use of systems able to stay for longer periods of time on the ocular surface due to interactions with ocular mucins (gel-forming and transmembrane mucins mainly), which are the major components on the preocular tear film (Fig 3). In this sense, the use of bioadhesive micro- and nanosystems offer a very promising approach to develop novel topical ophthalmic formulations.

Microparticles

Microparticles are solid drug carriers (1-1000 μm) capable of providing sustained and controlled release of loaded active agents. According to their structure, microparticles can receive the name of microcapsules (reservoir structure) and microspheres (matrix structure) (Fig 5).

The use of microparticles for topical therapies is not very common. One of the main reasons is their short contact time on the ocular surface. It is also important to note that the size of microparticles has to be controlled to be less than 10 μm to avoid possible eye irritation [2, 3].

Some authors have studied the use of polymeric biodegradable microparticles for ophthalmic topical administration. Addo et al. showed that the use of tetracaine-loaded microparticles (4 μm bovine serum albumin-chitosan microparticles, spray-drying method) increased the anesthetic effect of the drug up to 4-fold in rabbits, in comparison with a commercialized ophthalmic solution [4]. Other authors have examined the hypothesis that mucoadhesive microparticles may offer a means for slow drug release from microparticles that remain adherent to the ocular surface for an extended period of time. In that sense, Sensoy et al. demonstrated the utility of mucoadhesive polymers (hydroxypropylmethyl cellulose) for the preparation of bioadhesive microspheres loaded with sulfacetamide (2 μm polycarbophil microspheres, prepared by the spray-drying method) for the treatment of keratitis in rabbits [5]. Choy et al. observed that the use of a mucoadhesion promoter such as polyethyleneglycol (PEG), increased the microparticles' retention time (nile red-labeled polylactic-co-glycolic-PEG microparticles, <10 μm) up to 1 hour on the ocular surface of rabbits [6, 7]. Park et al. also used PEG as additive with PLGA microspheres loaded with brimonidine. The mucoadhesive microparticles increased drug bioavailability of the hypotensive agent in more than two folds compared to the marketed brimonidine formulation [8].

Nanoparticles

Nanoparticles are solid, submicron-sized drug carriers with diameters ranging from 1 to 1000 nm. Depending on their structure, they can be also classified into nanospheres and nanocápsulas (Fig 5). Because of their particle size, nanoparticulate systems offer the possibility to enhance the delivery and transport of drugs across ocular tissues. Nowadays it is possible to find studies in which drug-loaded nanoparticles prepared with biodegradable materials (such as PLA, PLGA, chitosan or albumin) demonstrate promising results.

Vega et al. showed that the topical administration of flurbiprofen-loaded nanoparticles (232-277 nm PLGA nanoparticles, prepared by the nanoprecipitation method) presented higher anti-inflammatory effect than a solution of sodium arachidonate, used as a reference, after topical instillation in rabbit eyes [9]. De Campos et al. studied cyclosporine A-loaded nanoparticles for topical administration (300 nm chitosan

nanoparticles, developed by ionic gelation technique). Chitosan is able to interact with ocular mucins and also open tight junctions of the epithelial cells, promoting the transcorneal flux. The system created improved the interaction of the drug with corneal and conjunctival tissues, finding therapeutic levels of cyclosporine at least during 24 or 48 hours post-administration in ocular surface tissues (conjunctiva and cornea, respectively) [10]. Chaiyasan et al. developed chitosan-dextran sulfate nanoparticles with a mean size of around 400 nm, as a topical ocular delivery system with lutein as a model drug (entrapment efficiency in the range of 60-76%). The positively charged nanoparticles prepared (+46 mV) resulted mucoadhesive and were considered suitable for drug delivery to the ocular surface [11]. In a different study, brimonidine-loaded nanoparticles were prepared by the ionic gelation technique in order to improve the bioavailability and to prolong the residence time of the formulation in the eye. Nanoparticles, with a mean particle size rage of about 270-370 nm (PI<0.5) showed an entrapment efficiency of the drug from 36-49%. In vivo studies showed a sustained effect of the formulation in comparison with conventional eye drops [12].

Liu et al. [13] demonstrated that the ocular delivery of baicalin (an anti-inflammatory and analgesic agent), could be enhanced by the use of solid lipid nanoparticles (91 nm, emulsification/ultrasonication method). These nanoparticles were able to enhance the corneal penetration of the drug in permeation studies performed in excised rabbit corneas. A significantly higher bioavailability of tobramycin was shown when 100 nm drug-loaded solid lipid nanoparticles were administered on the ocular surface in rabbit eyes. A significantly higher drug concentration in the aqueous humor was found in comparison with the same dose of a tobramycin aqueous solution [14]. A higher therapeutic efficacy and more prolonged effect was observed for methazolamide-loaded solid lipid nanoparticles when compared with a commercial formulation of the antiglaucoma active agent [15].

Hao et al. [16] prepared solid lipid nanoparticles within the colloidal range, dispersed in poloxamer-based hydrogels with thermosensitive *in situ* gelling properties (27.5 % poloxamer 407 and 3.55% poloxamer 188). *In vitro* corneal penetration revealed a nearly steady sustained drug release. Toxicity tests in the hen's egg test-chorioallantoic membrane showed no irritation, suggesting these systems as potential vehicles for ocular application. Wang et al. prepared diblock elastin-like polypeptide based nanoparticles with a multivalent presentation of lacritin at the surface (20–30 nm micelles at physiologically relevant temperatures)[17]. Thermo-responsive nanoparticles exhibited mitogenic activity in SV40-transduced human corneal epithelial cells. The *in vivo* efficacy was explored in wounds on the ocular surface of female non-

obese diabetic mice. Two doses of nanoparticles within 12 h after surgery produced significantly faster wound healing than controls. Histology analysis revealed that, after the treatment, no significant corneal inflammation was observed and the reconstituted ocular surface appeared as smooth as pre-procedure following 24 hours. This report provided the first *in vivo* verification of lacritin's wound healing potential and can be further applied to rationally bioengineer other peptide therapeutics into self-assembling nanoparticles for treating visual impairments.

Kao et al prepared a long-lasting pilocarpine-loaded chitosan/carbopol nanoparticle ophthalmic formulation that showed a slower release profile and the most significant long-lasting decrease in the pupil diameters of the rabbits, in comparison with those obtained with other systems such as a pilocarpine solution, a gel and liposomes [18]. The bioadhesive polymer hyaluronic acid has also been combined with dorzolamide and timolol loaded nanoparticles. This combination resulted very efficient at reducing intraocular pressure in rabbits in comparison with the same dose of a conventional solution of both hypotensive drugs [19].

The *in vivo* efficacy of cyclosporine A was also enhanced with the use of these systems [20]. Cationic nanoparticles were also employed to enhance the bioavailability of drugs, applied by topical administration, through electrostatic interactions between the negatively charged ocular surfaces and the positively charged formulation. According to this strategy, Başaran et al. [21] incorporated cyclosporine A into three different types of cationic chitosan nanoparticles. Authors employed low, medium and high molecular weight chitosan (20-200, 200-800 and 800-2000 cPs) to prepare nanoparticles following the spray-drying method (sizes between 318 and 433 nm). Medium molecular weight chitosan nanoparticles showed a more uniform release pattern and were used for the *in vivo* studies. A volume of 0.5 mL of dispersed nanoparticles (cyclosporine A content 10.58 ± 0.25% or 19.20 ± 0.33%) was applied in sheep eyes. Then, samples of aqueous humor and vitreous were collected at different time intervals for cyclosporine A quantification. Results showed a prolonged drug release from chitosan-based nanoparticles even after 72 hours with levels of 41.00±0.90 and 36.70±0.30 ng/mL in aqueous humor and vitreous samples, respectively.

Liposomes

Liposomes are spherical vesicles composed of an aqueous core enclosed by concentric phospholipid bilayers Fig 6. The size of liposomes ranges from 10 nm to 10 μm. According to their size and the number of lipid bilayers, liposomes are classified

into small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles, if more than one bilayer is present.

As well as nanoparticles, liposomal formulations have shown to increase drug residence time and to enhance drug permeation through corneal tissues, due to their size and surface properties. Positively charged liposomes, composed by cationic lipids such as dioleoyl-3-trimethyleammonium propane chloride (DOTAP) or stearylamine (SA), are able to interact with the anionic mucins present on the ocular surface (Fig 3), allowing the formation of a completely coated layer, increasing the contact time of the formulation and, subsequently, the drug bioavailability [22].

Schaeffer et al. reported a 4-fold increase of penicillin G bioavailability with the use of drug-loaded liposomes composed by phosphatidilcholine (PC), cholesterol (Cht) and SA prepared according to the thin-film hydration method, in comparison with the equivalent concentration of free drug [23]. Law et al. showed an increased residence time of acyclovir-loaded liposomes with the same components that promoted an enhanced bioavailability of the drug, in comparison with anionic liposomes, which were drained fast because they were not able to provide an electrostatic interaction with the ocular surface [24]. Also, Saettone et al. compared a positively charged liposomal acyclovir formulation (PC, Cht and SA, thin film hydration method) with a commercialized ointment. Liposomes increased acyclovir concentration in the aqueous humor of rabbits when compared with the ointment at the same drug concentration [25]. Acetazolamide and pilocarpine-loaded liposomes have also been investigated for the treatment of ocular hypertension, promoting a more pronounced and sustained reduction in intraocular pressure than conventional hypotensive solutions [26]. Li et al. investigated the potential of liposomes to enhance the local antiglaucomatous effect of brinzolamide [27]. *In vitro* release studies of brinzolamide-loaded liposomes (84.33±2.02 nm) displayed a biphasic release pattern with an initial burst release, followed by a second sustained release period. In Franz-type diffusion cells, corneal permeability resulted in a 6.2-fold increase in comparison with the commercial available formulation. *In vivo* studies in rabbits showed a more sustained and effective intraocular pressure reduction (5-10 mmHg) for drug-loaded liposomes. Chetoni et al. evaluated the *in vitro* and *in vivo* performance of a topical controlled release liposomal formulation containing distamycin A, for the treatment of acyclovir-resistant Herpes simplex virus keratitis [28]. The bioavailability of the drug in the tear fluid and the uptake into the cornea were increased after instillation of liposomes in rabbits, being less cytotoxic than distamycin A alone in rabbit corneal epithelial cells. Distamycin

concentration in cornea, reached IC50 values against HSV, without any sign of transcorneal permeation of the drug.

Several studies have shown that hybrid systems (liposomes combined with hydrogels) can increase the residence time of the formulations, thus prolonging the drug release rate [29]. Budai et al. encapsulated ciprofloxacin in multillamelar vesicles that were suspended in different polymeric solutions forming hydrogels, such as polyvinyl alcohol 0.14% or polymethacrylic acid 0.1%. The release half-time of ciprofloxacin was increased from 72 min (ciprofloxacin hydrogels without liposomes) to 212 min and 644 min when lecithin or α-l-dipalmithoyl-phosphatidylcholine lipids were used to prepare the vesicles, respectively [30]. Liposomes coating has been demonstrated to increase the release rate of ciprofloxatin up to 10 h (75% of the dose) using 1.5% Carbopol® 940 [31-33]. Additionally, authors observed that the transcorneal permeation of ciprofloxacin was augmented 5 times with the use of carbopol-covered liposomes, in comparison with uncovered liposomes (3-folds increment) or a ciprofloxacin solution used as a reference[34]. The use of chitosan to form hydrogels to cover drug-loaded liposomes has also showed promising results. Hosny et al. [35] prepared oxoflacin-loaded liposomes dispersed in a thermosensitive gel composed by chitosan and β-glycerophosphate salt (1.8% w/v and 3.8% w/v respectively). The presence of the hydrogel increased the amount of drug permeated through the cornea in 7-fold, in comparison to the drug aqueous solution at the same concentration. A different interesting technological strategy to achieve higher concentrations of the therapeutic agent in ocular tissues involves the dispersion of drug-loaded liposomes in collagen shields. Pleyer et al. employed this strategy in rabbit eyes. As expected, they found that cyclosporine levels in cornea, sclera, aqueous humor and vitreous were significantly higher in these animals, in comparison with conventional cyclosporine eye drops [36]. Also, the combination of liposomes and bioadhesive polymers such as carboxymethyl cellulose or hyaluronic acid increased the hypotensive effect of a melatonin analogue 5-MCA-NAT (maximum intraocular pressure reduction of 36.72±2.77% and 39.13±2.21% respectively) in rabbit eyes compared to other liposomes formulated without polymers (29.44±2.40%). This hybrid formulation was able to increase the bioavailability of the therapeutic agent (the effect lasted more than 8 hours), being soft with the ocular surface. As the novel formulation simulate tear film composition, it might relieve symptoms of secondary diseases affecting the ocular surface derived from the chronic use of antiglaucoma topical medications [37]. In a different approach, Dong et al. designed and prepared ibuprofen-loaded liposomes coated by regenerated silk fibroins, which are novel adhesive excipients with different

dissolving times [38]. No detectable toxicity was observed in cells after 12 hours of exposure to silk fibroins. The novel liposomes showed sustained release and *in vitro* corneal permeation of ibuprofen as compared to a drug solution and liposomes without silk fibroins.

Microemulsions

Microemulsions are pharmaceutical systems composed of an oily phase, an aqueous phase and a combination of surfactant and co-surfactant at appropriate ratios to stabilize the system (Fig 7). Microemulsions possess optimal characteristics to be administered by topical route. Depending on their charge and viscosity, the retention time onto the ocular surface can be increased allowing the delivery and permeation of drugs across the ocular surface.

Klang et al. demonstrated that positively-charged microemulsions were able to enhance drug corneal penetration, achieving higher concentrations of drug (indomethacin) in aqueous humor and sclera-retina in comparison to negatively-charged microemulsions, due to the electrostatic effect between the cationic formulation and the anionic cornea. Moreover, positively-charged microemulsions (composed by poloxamer, stearylamine, lipoid 80 and α-tocopherol) resulted well tolerated by animals, since no signs of inflammation or irritation were detected after its administration during eight consecutive hours (1 adm/h) [39]. The use of bioadhesive polymers in the aqueous phase of the microemulsions, has demonstrated to enhance the drug bioavailability as well. In that sense, Chauhan et al. developed timolol microemulsions in HEMA (2-hydroxyethyl methacrylate) to increase the ocular residence time of formulations, with promising results [40]. Phase-transition microemulsions have also been studied for ophthalmic drug delivery purposes. In these microemulsions, there is a phase change after their instillation that produces a viscosity increment and a flow rheological change (from newtonian to pseudoplastic), increasing the ocular residence time of formulations on the ocular surface [41, 42].

Niosomes

Niosomes are nonionic surfactant vesicular systems formed from the self-assembly of non-ionic amphiphilic compounds in aqueous media, resulting in bilayer vesicles of 10-0.5 μm in size (Fig 7). They have gained popularity in ocular drug delivery because they can provide prolonged duration of action at the corneal surface and increase the ocular bioavailability of ophthalmic therapeutics.

The use of these systems has shown promising results for the treatment of ocular hypertension associated to glaucoma. Aggarwal et al. reported that acetazolamide-loaded cationic niosomes provided good entrapment efficiency and corneal permeability, being effective for the reduction of intraocular pressure in experimental animals. However, positively charged niosomes, composed of sorbitan esther, cholesterol and SA, resulted toxic for corneal cells. In order to reduce corneal toxicity of niosomes, a bioadhesive niosomal formulation of acetazolamide was also prepared by coating niosomes with Carbopol®. The bioadhesive formulation was less toxic that non-coated vesicles, and showed a 2-folds increment in the duration of action in comparison with a commercial formulation [43]. In a different study, authors observed that the niosomal formulation induced a more rapid penetration of the drug across the cornea and more intraocular pressure reduction in the contralateral eye in comparison with a conventional hypotensive solution [44], indicating lesser systemic side effects. Abdelkader et al. developed naltrexone hydrochloride-loaded niosomes and tested the incorporation of different additives to the formulation, to demonstrate that these compounds can influence the entrapment efficacy and also the size and shape of niosomes [45]. *Ex vivo* transcorneal permeation studies conducted using excised cow corneas showed that niosomes were capable of controlling drug permeation and enhancing its corneal permeability. These formulations resulted practically non-irritant [46]. The same authors observed that niosomal preparations were able to protect the encapsulated drug from the photo-induced oxidation up to 3-folds in comparison to free naltrexone solutions [47].

Li et al. developed proniosome-derived niosomes for topical ophthalmic delivery of tacrolimus [48]. Freshly excised rabbit corneas were employed to analyze the *in vitro* permeation of tacrolimus from the vesicles and the drug retention in the cornea. Results showed a significant increase in tacrolimus permeation as compared with commercial ointments. The *in vivo* ocular irritation test of 0.1% tacrolimus-loaded niosomes showed no irritation and good biocompatibility with cornea after 21 consecutive days of instillations (4 times/day). The *in vivo* anti-allograft rejection assessment was performed in a rat corneal xenotransplantation model. The results showed that tacrolimus-loaded niosomes delayed the occurrence of corneal allograft rejection and significantly prolonged the median survival time of corneal allografts, in comparison to cyclosporine eye drops, drug-free niosomes, or untreated eyes. Abu et al. reported the preparation and characterization of an ocular niosomal/hydrogel formulation containing 0.5% atenolol for the treatment of ocular hypertension associated to glaucoma. Niosomes were prepared with span 60 and cholesterol at

different molar ratios. The size of niosomal formulations ranged from 56.5 to 155.3 nm and showed a high retention of atenolol inside vesicles up to a period of 3 months at 4ºC (83.1±2.3%). Examination of rabbit eyes did not show irritation signs. Atenolol niosome/carbopol gel showed slower and sustained *in vitro* release of atenolol in comparison to the rest of formulations, and a maximum IOP reduction of 49.80% after 5.33h of drug administration. The effect was sustained and prolonged for 8 hours.

Dendrimers

The term “dendrimer” arises from the Greek Dendron, meaning “tree”, and meros, meaning “part”. It graphically describes the structure of this class of synthetic macromolecules that have highly branched, three-dimensional features that resemble the architecture of a tree (Fig 8). Despite their large molecular size (5,000-500,000 g/mol), they are structurally well-defined and have a low polydispersity [49].

Mucoadhesive dendrimers could be used to increase the drug retention time on the ocular surface after topical administration, with the additional advantage that they do not provoke blurred vision nor formation of any veil on the corneal region, problems typically associated with other bioadhesive polymers [50]. This idea was firstly explored by Vandamme and Brobeck [51]. They tested several families of PAMAM dendrimers in combination with pilocarpine nitrate and tropicamide as model drugs (miotic and mydriatic activity respectively) in New Zealand albino rabbits. No ocular irritation or watering reflex was observed in any of the dendrimers evaluated (concentrations up to 2.0% w/v), after instillation. The residence time of PAMAM dendrimer with amino surface groups was even significantly longer than that of Carbopol® or HPMC solutions. During the evaluation of dendrimer-drug interaction, a host–guest relationship was suggested leading to a sustained release of both drugs. The in vivo results obtained showed that the co-administration of PAMAM dendrimers with these drugs prolonged their pharmacological effect, indicating an increment in the precorneal residence time. This aspect was deeply evaluated by Bravo-Osuna et al. [52]. These authors developed the first *in vitro* method specifically designed to evaluate mucoadhesion with ocular mucins. The technique developed, based on biosensor chip technology, was able to evaluate the interfacial interaction phenomena between human transmembrane ocular mucins and dendrimers. A strong tendency to originate interfacial interaction with human ocular transmembrane mucins was observed for PAMAM dendrimers compared to classical polymers typically used to increase the drug retention time on the ocular surface (hyaluronic acid, carboxymethyl cellulose and hydroxypropylmethyl cellulose), showing a relevant importance of the co-operative

effect of dendrimer due to the proximity of the active chemical groups and the high importance of non-ionic interactions at that level.

Yao et al. evaluated the potential of PAMAM complexes with puerarin as corneal penetration enhancement agents using the Valia-Chien diffusion cell with excised rabbit corneas. [53, 54]. The enhancing permeation behaviour of PAMAM dendrimers was observed probably due to their interaction with corneal epithelium membrane cells or originated by their ability to relax the epithelium cell junctions to increase the drug flux. PAMAM Dendrimers have been also explored as gel-forming tool to increase the residence time of drugs or even other drug delivery systems on the ocular surface. Holden et al prepared PAMAM-PEG cross-linked gels able to enhance the corneal transport of two antiglaucoma drugs (brimonidine and timolol maleate) up to 4.6 folds in comparison to drug solutions [55]. When these drugs were previously encapsulated in PLGA nanoparticles and suspended in PAMAM gels the hypotensive effect was *in vivo* detected in the aqueous humor and cornea up to seven days after instillation in Dutch-belted rabbits [56].

Other dendrimers have been also tested at ocular topical level. Spataro et al. developed an interesting new series of phosphorus-containing dendrimers [57]. The dendrimers were used to form electrostatic complexes with carteolol, an ocular anti-hypertensive drug used to reduce the intraocular pressure in glaucoma treatment. Complexes were instilled in rabbits' eyes to evaluate the benefit of these nanosystems to enhance the penetration of the drug to the aqueous humor. The quantity of carteolol penetrating inside the eye was larger (2.5 folds) than expected when compared with carteolol alone. In another interesting work, Durairaj and co-workers evaluated the use of complexes formed by the combination of dendrimeric polyguanidilyated translocators (DPTs) and gatifloxacin in the treatment of bacterial conjunctivitis [58, 59]. The complexes formed nanostructures (346 nm). It was observed that the complexes rapidly penetrated into the corneal cell in a few minutes, in comparison to drug alone. The nanosized complex was instilled in the eye of New Zealand rabbits, showing an increment in the bioavailability of the drug in targeted tissues in conjunctiva and cornea, when compared with drug solution. Authors suggested that the systems created would allow the instillation of the drug in the complex only once a day to control the infection. Mishra and Jain also observed an increment in drug bioavailability after instillation in New Zealand rabbits of nanostructures formed by the combination of acetazolamide with G5 poly(propyleneimine dendrimers (PPI). The reduction of the intraocular pressure was sustained for almost 4 hours for the dendrimer-based formulation, while pharmacological effect was observed only up two hours when plan

drug solution was used. Author suggested that the interaction of dendrimer formulation with the corneal surface might form a structure that leads to the entrapment of more amount of the instilled dose [60].

INTRAOCULAR ADMINISTRATION

Intraocular administration covers several routes that target the anterior and/or posterior segment of the eye, such as intracameral, intravitreal or subretinal injections (Fig 1). Intraocular drug delivery refers to the release of the therapeutic agents into the eye, in some cases targeted directly at the site of action. In this way, drugs diffuse locally, reducing the delivery to other non-intended sites of action and the potential side effects produced.

The intracameral administration is exclusively used to describe the injection of formulations into the anterior segment of the eye. However, this route does not allow delivering significant concentrations of drugs to the posterior segment, so it is generally used to prevent endophthalmitis by delivering antibiotics after cataract surgery. Some authors have attempted to use polymeric drug delivery systems as sustained-release formulations into the anterior segment, but they can cause physical clogging of the aqueous outflow and consequently increase intraocular pressure.

The intravitreal injection is the most efficient route to treat posterior segment disorders. In comparison to topical and systemic administrations, this route presents clear advantages, assuring a very low prevalence of systemic side effects. However a number of disadvantages are associated with the intravitreal administration. First of all, it is an invasive route that breaks the ocular tissues compromising the immunity privilege of the eye. Furthermore, as the drug is locally deposited, due to the initial high concentrations that are achieved after the injection, a retinal toxicity can be produced. Although the clearance from the vitreous depends on the lipophilicity and molecular weight of the active substance, this process is usually fast. In several cases, drug elimination occurs in a few hours. Moreover, due to the fact that most of posterior segment diseases are chronic, repeated injections are needed even for active substances presenting long half-life values. Besides, frequent repeated injections increases the risk of endophthalmitis, damage to lens or retinal detachment, among others. So the general objective in the development of new intraocular therapies for chronic diseases is to maintain drug concentrations within the therapeutic window at the target site and reduce the frequency of administration. In order to reach these objectives, several authors have explored the use of micro- and nanoscale systems for the treatment of posterior segment diseases. Microsystems have demonstrated to be

useful tools in long-term therapies. They can control the release of the active substance for weeks, even months, reducing the frequency of injections and maintaining sustained drug concentrations in the vitreous. The utility of nanosystems for the treatment of posterior segment diseases is more directed to gene delivery and to the protection of the active substances from the external environment to be targeted to damage ocular tissues. This strategy is especially important when bioengineered compounds are used, such as peptides, proteins or nucleic acids.

The usefulness of subretinal injections to treat alterations in the retina is clear; however, the risk of this administration is evident and is still being tested in a preclinical setting [61].

Microparticles

The main advantage on the use of microparticles for intraocular administrations is that they can be injected in the form of suspensions by using conventional needles. Microparticles are typically dispersed in a physiological vehicle (phosphate buffer or balanced salt solutions), but sometimes viscous vehicles, such as hyaluronic acid or hydroxypropylmethyl cellulose, are used to improve the injectability of the suspensions [62].

Many authors have tested the use of microparticles as drug carriers in the last decades. Posterior segment diseases such as proliferative vitreoretinopathy, age-related macular degeneration, diabetic retinopathy, uveitis, macular edema, citomegalovirus, glaucoma and retinitis pigmentosa have been treated with drug-loaded microspheres with a relatively high rate of success [63]. For intraocular administration, a large variety of active substances have been included in microspheres (i.e. antiproliferatives, antiinflammatories, immunosuppressants, antibiotics and even biotechnological therapeutic agents). Microparticulate systems have been prepared from biodegradable polymers such as gelatin, albumin, polyorthoesters, polyanhydrides and polyesters. Among them, the poly (lactic) acid (PLA) and poly (glycolic) acid (PGA) polymers and their copolymers poly (lactic-co-glycolic) acid (PLGA) are the most employed. The use of these biomaterials in the clinical practice has been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). For ocular application, and especially for the treatment of diseases affecting the back of the eye, these erodible polymers have been employed to prepare different devices (implants, scleral plugs, pellets, discs, films, rods, nanoparticles and microparticles) [64].

An interesting work from Moritera et al. showed the administration of adryamicin-loaded microspheres (50 μm particle size) in an animal model of proliferative vitreoretinopathy. Authors found that, not also the retinal detachment was decreased from 50% to 10% in the rabbits after 4 weeks of the intravitreal injection, but a significant decrease in the retinal toxicity what also produced, when comparing a single injection of microspheres with the administration of the same amount of drug in solution [65, 66]. The antiviral effect of ganciclovir-loaded microspheres was evaluated by Veloso et al. in infected rabbits' eyes [62]. In treated eyes, vitritis, retinitis and optic neuritis decreased during the 14 days of the study, in contrast to control eyes. Histopathology analysis showed minimal focal disruption of the retinal architecture in eyes injected with drug-loaded microspheres. After 8 weeks, no adverse tissue reaction was clinically or histopathologically observed in the injected eyes. Conti et al. developed PLA and PLGA microparticles loaded with acyclovir using the spray-drying technique. After 14 days, the active substance was detected in the vitreous of rabbits receiving D,L-PLA (28,000 g/mol) microparticles (0.5 mg) [67]. Duvvuri et al. dispersed ganciclovir-loaded microspheres in a thermogellin PLGA-PEG-PLGA solution that was then injected in rabbit eyes. After the injection, drug levels in the vitreous were significantly higher and maintained for a prolonged time in comparison with a drug solution (14 days versus 54 hours, respectively) [68].

The treatment of intraocular inflammation, uveitis or intraocular immune disorders with microparticles has also been extensively investigated. Cyclosporine-loaded PLGA 75:25 microparticles (50 μm particle size) prepared by He Y et al. [69] produced a 10-times increment in the residence time of the drug in comparison with a cyclosporine solution. Microspheres were also able to maintain therapeutic concentrations for at least 65 days in the disease-related tissues such as the choroid-retina and iris-cilliary body. Dexamethasone-loaded PLGA 50:50 microspheres (53 μm) have been tested for the prevention of ocular inflammation in an animal model of uveitis provoked by lipopolysaccharide injection [70]. Authors showed that the intraocular inflammation was significantly reduced in animals receiving drug-loaded microspheres in comparison to control (blank microspheres), even when a secondary uveitis was simulated with a second injection of lipopolysaccharide 30 days after microspheres' injection.

Some studies performed with microspheres loaded with anti-inflammatory drugs have already been performed in humans. For example, Cardillo and co-workers [71] reported human studies of the intravitreal injection of triamcinolone-loaded PLGA microspheres. Nine volunteer patients suffering diffuse macular edema were treated with the microspheres and the efficacy of the formulation was compared to conventional

triamcinolone injections. Interestingly, the eyes treated with triamcinolone microspheres showed a marked decrease of retinal thickness as well as an improved visual acuity for about 12 months.

The development of new bioengineered products such as peptide, proteins or nucleic acids, for the treatment of ophthalmic diseases has made necessary to design new microparticulate systems able to effectively administrate such molecules in the target site. This strategy has been employed for the treatment of glaucoma. The therapeutic approach focuses on promoting the survival of retinal ganglion cells (RGCs) (Fig 4). In that sense, Andrieu-Soler et al. [72] developed PLGA microspheres loaded with GDNF, a glial-cell-line-derived neurothropic factor, that resulted efficient at preventing the retinal degeneration in a rd1 mouse model for 17 days. The efficacy of GDNF-loaded microspheres has also been tested in a glaucoma rat model based on the intraocular pressure elevation with an episcleral injection of a hypertonic NaCl solution, showing a significant increase of the survival of retinal ganglion cells and their axons 9 weeks after the intravitreal injection of the microspheres [73]. Checa-Casalengua et al. [74] developed PLGA microparticles (20-40 μm) loaded with an antioxidant (Vitamin E) and GDNF. The multi-loaded microparticulate systems were able to release the protein in its bioactive form for more than 130 days. Afterwards, authors observed that a single injection of these microspheres (25 μg) in a rat model of glaucoma based on intraocular pressure elevation, were enough to promote a retinal ganglion cells survival of 72.7% eleven weeks after injection, in contrast to 36.6%, 30.6%, and 29.0% of RGC survival observed in eyes treated with GDNF in solution, vitamin E and blank microspheres respectively.

Andrieu-Soler et al. also studied the efficacy of PLGA microspheres (27 μm) loaded with recombinant human glial cell line-derived neurotrophic factor (rhGDNF) in preventing retinal degeneration in an animal model (mice) of retinosis (rd1) for 17 days [72]. Authors described a significant delay of rod photoreceptor degeneration in the animals receiving the rhGDNF-loaded microspheres in comparison to control group (untreated animals) or the group receiving blank microspheres. Retrobulbar administration of PLGA microspheres loaded with the IKK2 inhibitor, 2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide TPCA-1, promoted a significant reduction of a laser induced choroidal neovascularization (CNV). The effects were observed at seven days after laser injury in mice. The authors suggested a potential clinical interest of the TPCA-1 microparticulate system in the treatment of CNV in patients with wet age related macular degeneration and other retinal neovascularization pathologies [75]. PLGA microspheres prepared by electrospray for

the release of ranibizumab by intraocular administration, have been reported by Zhang et al. The proposed encapsulation method provided a minimal loss of bioactivity of the antivascular endothelial growth factor. After intravitreal injection of the microparticles in a chick model, the active molecule was released in a controlled fashion for longer than one month. Interestingly, no significant reaction or cell death was observed after intravitreal injection of 0.2 mg of particles [76].

The utility of microparticles in rare ocular diseases, such as retinitis pigmentosa, has also been evaluated. PLGA microspheres (2-40 μm) loaded with tauroursodeoxycholic acid (TUDCA) and phosphate solution (control) were intravitreally injected in homozygous P23H line 20 days old rats. After assessing higher retinal functionality at different postnatal days (P80, P100 and P120) in the TUDCA treated animals the authors suggested the potential of these formulations to delay vision loss in retinitis pigmentosa [77].

Nanoparticles

Advances have already been performed in the intravitreal and subretinal administration of nanoparticles. Robinson et al. evaluated the efficacy of AG1478-loaded PLGA nanoparticles (359 ± 54 nm) by intravitreal injections in a rat optic nerve crush injury model [78]. Four weeks after the injection, authors were able to detect NPs in the vitreous and could find fiber growth through the injury site. In a different study, the molecule tetra-iodothyroacetic acid, able to block angiogenic action over VEGF and erythropoietin, was encapsulated in PLGA nanoparticles, and its effect was evaluated in mice. While the administration of the drug alone had no statistically significant effect on retinal neovascularization compared with the vehicle, the administration of a single injection of loaded-NPs significantly reduced retinal neovascularization by 30.9% three days after injection [79]. An expression plasmid of plasminogen kringle (K5), a natural angiogenic inhibitor, was encapsulated in PLGA: chitosan NPs (260 ± 30 nm) and injected into the vitreous in a rats. Results showed that, for at least four weeks, K5-NPs attenuated vascular endothelial growth factor and reduced leukostasis and vascular leakage in the rat model used [80]. Farjo et al. determined the efficiency of compacted DNA nanoparticles to transfer a plasmid encoding the green fluorescent protein (EGFP). Nanoparticles were administered by subretinal injection (1 μl) in mice. 2 days post-injection the examination a fluorescent signal in the retina inner plexiform layer and in retinal ganglion cells was detected. Substantial EGF fluorescence was also detected in the lens, cornea and trabecular meshwork with no evidence of cellular infiltration or inflammation [81]. Other authors studied the therapeutic efficacy and

safety of compacted DNA-NPs gene delivery into the subretinal space of a juvenile mouse model of retinitis pigmentosa. Electroretinography studies showed a modest but statistically significant improvement in rod function in comparison to controls after the injections [61]. Cai et al. determined the utility of small nanoparticles (diameter < 8 nm) composed of a therapeutic gen in a mouse model of retinitis pigmentosa with a phenotype of slow retinal degeneration. In this work, NPs were compacted by a peptide with a high transfection activity (CK30PEG, a PEG substituted 30-mer lysine peptide). The efficacy of the system was evaluated in terms of its ability to rescue the disease phenotype in the animal model, measuring the levels of several photoreceptor-specific proteins typically reduced in these animals. Nearly all of the photoreceptor population were transfected by the subretinal delivery of nanoparticles. Additionally, a cone function restoration to almost normal levels and a rod function improvement was observed in treated animals in comparison to control [82]. Mitra et al. formulated, characterized and tested glycol -chitosan nanoparticles for gene delivery to the eye [83]. pscCBA-GFPA (pDNA), known to express green fluorescent protein (GFP), was compacted with glycol-chitosan nanoparticles. In order to assess the ability of nanoparticles to drive ocular gene expression *in vivo*, particles were subretinally injected in wild-type albino mice. Authors observed GFP expression in nanoparticles but not pDNA alone. GFP expression was primarily localized to the region of injection. This study showed that the pDNA was stable inside the non-viral system and retained its native conformation and functional integrity, facilitating gene expression in the retinal pigment epithelium after subretinal delivery.

Liposomes

As well as nanoparticulate systems, liposomal formulations in intraocular drug delivery are intended to target and protect the active substance and improving the therapeutic effect. Liposomes are able to improve drug stability in biologic fluids and can reduce retinal toxicity [84]. However, they have limited drug capacity and are difficult to sterilize, making it difficult to develop liposomal formulations for intraocular drug delivery.

Several drugs with known high toxicity have been successfully formulated as liposomal formulations. A clear example is amphotericin B, which produced less toxic effects than a marketed solution after a single intravitreal injection in healthy pigmented rabbits [85, 86]. In a different study, an acyclovir pro-drug called HDP-P-GCV, was entrapped into liposomes and injected into the vitreous in an animal model of cytomegalovirus retinitis, being able to protect the treated eyes against the virus for 4-6 weeks [87].

Several authors have evaluated the efficacy of liposomes to encapsulate DNA or RNA for gene therapy. However, these lipoplexes have to overcome two important limitations. Firstly, the vitreous is a high viscosity material that induces lipoplexes' aggregation and is able to avoid the diffusion of lipoplexes to the site of action. Secondly, the presence of the polyanionic glycoaminoglycans in the vitreous can displace the DNA from the complex. In order to minimize these limitations, Peeters et al. developed PEGylated lipoplexes (1,2-Distearoyl-sn-glycero-3 phosphatidylethanolamine -PEG) that showed a decreased surface charge in comparison to non-PEGylated lipoplexes and an increased transport to the retinal pigment epithelium [88]. Bochot et al. also developed PEGylated liposomes for the intravitreal delivery of an oligonucleotide model, pdT16. The liposomal formulation (PC:Cht:PEG-Polyethylene glycol-distearoyl ethyl phosphocoline) was able to protect the active substance against degradation in the vitreous and to prolong the pdT16 release into the vitreous, retina and choroid [267]. Lajavardi et al. also used PEGylated liposomes to improved intravitreal delivery of a proteic substance, VIP (vasointestinal peptide) for the treatment of uveitis. Labelled rhodamine (Rh) liposomes (PC:PG:Cht:PEG-Polyethylene glycol-distearoyl ethyl phosphocoline:PE-Rh), dispersed in hyaluronic acid 1.2% were able to protect the protein from degradation in the vitreous and to maintain a sustained release of VIP [89].

The potential of liposomes to deliver functional proteins in retinal photoreceptors and modulate their physiological response has recently been investigated by Asteriti et al. Vesicles, prepared with L-α-phosphatidylcholine and colesterol (8:10), were loaded with rhodamine B, recombinant myristoylated recoverin (an endogenous protein regulating the duration of the phototransduction cascade in photoreceptor cells) or antibodies against recoverin [90]. The efficacy of liposomes was tested in isolated mouse retinas by electrophysiology. The *in vivo* retinal biodistribution was evaluated by intravitreal injection (1 µL) in mice. Electrophysiological experiments showed that temperature seemed to be an important factor influencing the fusion of the vesicles with membranes. If the experiment was performed at 22ºC, no significant differences between treated and untreated retinas could be noticed, while a modification to the phototransduction cascade was produced when the experiment was performed at body temperature, due to a fusion of the vesicle within the photoreceptor layer.

Dendrimers

Intravitreal injection of drug delivery systems based on dendrimers has been explored in several works. Wimmer et al. prepared lipid–Lys dendrimers able to promote the

ODN-1 (anti-VEGF activity) delivery into the nuclei of retinal pigmented epithelium cells (RPE D407) cultures [91]. These new dendritic structure combined the presence of the lipidic part, that might facilitates their crossing of biological membranes, and the poly-Lys part that could interact with ODN-1 protecting it against nucleases. The chemical family of these lipid–Lys dendrimers was subsequently increased and their utility *in vivo* was evaluated showing reduction in VEGF expression (40-60% during the first 24 h) after intravitreous injection in choroidal neovascularisation animal models (rats) [92]. Interestingly, some of the prototypes evaluated extended the antiangiogenic effect up to 4 months. No significant toxicity and damage were observed on injected rat eyes after ophthalmological examinations[93]. The presence of galactose moieties in the lipid-Lys dendrimers structure increased their solubility and their transfection efficiency in RPE 51 cells [94].

Izze et al. demonstrated that the combination of PAMAM-OH dendrimers with fluocinolone acetonide was able to release the drug in a sustained manner for 90 days. *In vivo* efficacy studies demonstrated that only one intravitreal injection (1 µg of drug/ 7 µg of dendrimer) was able to stop retinal degeneration, preserve photoreceptor outer nuclear cell counts, and attenuate activated microglia, for an entire month in a rat retinal degeneration model. According to authors, this pathology-dependent biodistribution could be exploited to treat disease promoting retinal neuroinflammation like age-related macular degeneration and retinitis pigmentosa [95]. In this sense, other authors have also explored the use of PAMAM-OH dendrimers for the intracellular delivery of anti-inflammatory drugs at retinal level. Kambhampati et al. evaluated the *in vitro* cell uptake of triamcinolone acetonide conjugated with PAMAM-OH dendrimers in microglial and retinal pigmented epithelium [96]. The nanosystems created were able to reduce the inflammatory activity in microglia cells for three days after 12 hours treatment. Conjugated dendrimers also promoted a reduction in VEGF production in hypoxic RPE cells 100-folds higher than the same amount of drug alone. Authors suggested that the combination of the drug with the dendrimer not only improved its therapeutic effect but also reduced its toxicity. They postulated these conjugated as useful strategy in the treatment of ocular diseases characterized by neuroinflammation and neovascularization such as age-related macular degeneration or diabetic retinopathy among others. Hennig et al. prepared PEGylated PAMAM dendrimers conjugated with angiotensin receptor blockers demonstrating that this combination increased the receptor affinity in 6 folds [97]. This fact could be also interesting in the treatment of ocular diseases accompanied by retinal neovascularization, however, to our knowledge, no *in vivo* studies have been yet performed in this sense.

PERIOCULAR AND SUPRACHOROIDAL ADMINISTRATION

Periocular administration involves peribulbar, posterior juxtascleral, retrobulbar, sub-tenon and subconjunctival routes (Fig 1). Depending on the site of administration, a drug can access target sites in the posterior segment through the sclera, the choroid and/or aqueous humor and vitreous. However, the lymphatic flow in conjunctiva and episclera and/or the blood flow of conjunctiva and choroid, help eliminate the drug very fast from the administration site, limiting the access of these molecules to the intraocular tissues, even if they are macromolecules. Periocular administration is a less invasive and potentially safer alternative than intraocular injections, and the interest of this route is directed to the anterior segment of the eye (anti-inflammatory agents and anaesthetics by subconjunctival injection) [98] or to deliver drugs to the outer retina (photoreceptors and RPE) when it has been demonstrated to be more effective than intravitreal injections [99-101].

Microparticles

In order to provide sustained drug levels and enhanced drug absorption into the intraocular tissues, periocularly administered microparticles have to be maintained in the site of administration for a long time. However, particle size may limit the therapeutic effectiveness of these systems, by influencing their clearance by the systemic and lymphatic circulation. In this context, Amrite et al. studied the effect of the particle size disposition of non-biodegradable nanoparticles (20 and 200 nm) and microparticles (2 μm). In that study, only 200 nm and 2 μm particles stayed at the site of administration for at least 60 days, while 200 nm particles were rapidly cleared [102, 103]. Kompella et al. studied the delivery of anti-inflammatory drugs from micro- and nanoparticles by periocular administration. Budesonide-loaded PLA particles (345 nm and 3.60 μm particle sizes) were subconjunctivally injected in rats and the ocular tissues levels of the drug were analysed at different times in retina, vitreous, lens and cornea. On day 1, drug levels in the nanoparticle group were higher in all the tissues that were evaluated, when results were compared with the group receiving microparticles. However, the opposite situation took place on days 7 and 14, showing the potential utility on biodegradable microparticles in sustained retinal drug delivery [104]. Ayalasomayajula et al. studied the potential usefulness of subconjunctival injections of celecoxib-loaded PLGA 85:15 microparticles (3.9 μm) in the treatment of diabetes-induced retinal abnormalities. In a rat model of diabetes-induced retinal oxidative stress, microparticles were able to release the drug in a sustained manner

during 14 days in retina, vitreous, lens and cornea. Furthermore, the diabetes was significantly reduced [105]. In a different work, celecoxib-loaded microparticles were able to delay the development or progression of the early pathopysiological changes in the retina in a streptozotocin diabetic rat model. Celecoxib-microparticles significantly reduced the diabetes-induced elevations of prostaglandin E2 (PGE2) secretion, VEGF , the vitreous-plasma protein ratio and the blood-retinal barrier leakage without causing any damage to the retina[106].

The effect of the subconjuntival injection of adriamycin-loaded PLA microspheres in the prevention of post surgical fibrosis after glaucoma filtering surgery was evaluated by Kimura et al. The results showed an improvement in intraocular pressure reduction and filtering bleb survival in the treated eye in comparison to the control eyes, up to 12 days after the injection [107]. Gomes do Santos et al. prepared nanosized complexes of antisense TGF-β2 phosphorothioate oligonucleotides (PS-ODN) with and without polyethylenimine (PEI) encapsulated in PLGA microspheres ("Trojan" microspheres) to be injected in a rabbit experimental model of filtering glaucoma surgery. Results showed a significant increase in the intracellular penetration of ODN in conjunctival cells and demonstrated a 100% bleb survival. The subconjunctival injection prevented post-surgical fibrosis following trabeculectomy for 42 days[108].

The utility of PLGA/glucose microspheres loaded with PKC412 has also been evaluated by Saishin et al. in a porcine model of laser-induced choroidal neovascularization. The subconjunctival injection of these particles caused a significant suppression of the neovascularization development at rupture sites in Bruch's membrane and, twenty days after injection, drug levels were detectable in vitreous, retina and choroid, indicating the penetration of the drug across different tissues [109]. In this context, a different approach studied the use of anti-VEGF RNA aptamer-loaded PLGA microspheres [110]. The microparticles were able to deliver the aptamer in a sustained manner and the released aptamer retained its bioactivity, according to its antiangiogenic effect in human umbilical vein endothelial cells. After *in vitro* permeation study it was observed that microparticles were able to release the aptamer through the sclera for a 6-days period.

Not only subconjunctival, but other periocular routes have been also evaluated. Interesting results have been obtained by administering drug-loaded microparticles in the sub-tenon virtual space. Panganelli et al. demonstrated the utility of the sub-tenon injection of ciprofloxacin-loaded microspheres combined with triamcinolone in the prevention of the ocular inflammation and infection after cataract surgery. Authors

compared this treatment with the topical administration of prednisolone (1%) and ciprofloxacin (3%) eye drops during 28 days. Both treatments were evaluated in terms of efficacy (anterior chamber cell and flare, conjunctival erythema, ciliary flush or symptoms of ocular inflammation) and safety (intraocular pressure, biomicroscopy and ophthalmoscopic findings). Results demonstrated that a single periocular injection had same therapeutic response and ocular tolerance as continuous administration of eye drops in controlling inflammations after cataract surgery [111].

The tolerance of fluorescent nanoparticles (20 nm and 500 nm) and microspheres (1 mm and 10 mm) after suprachoroidal administration was studied by Patel et al. Authors described a normal fundus of the injected rabbit eyes with no inflammation or abnormalities as compared with uninjected eyes [112]. An interesting study tested the localization of microparticles and nanoparticles in the site of injection after administration in the suprachoroidal space using microneedles [113]. To this, non-biodegradable fluorescent particles were deposited in the suprachoroidal space using different solutions. When suspended in saline solutions, the particles were distributed over 29-42% of the suprachoroidal space. The larger spreading (100%) was observed for moderately non-Newtonian vehicles. Particles were localized at the site of injection for strong non-Newtonian polymer solutions.

The combination of silicon microparticles with electrospun polycaprolactone (PCL) fibers was designed to support mammalian cells providing a substantial surface area of efficient delivery of adsorbed active substances. In this novel implantable scaffold, particles of different sizes (150-250 μm or <40 μm) pressed into the fibers made by PCL. After implantation under the conjunctiva, the selected formulation did not produce significant neovascularization bur promoted a macrophage and mild foreign body response [114].

Nanoparticles

As mentioned previously, the therapeutic utility of periocularly administered nanoparticles is limited by their size. Only particles sizes larger than 200 nm are able to remain in the administration site [103, 115].

Kompella et al. evaluated the effect of subconjunctivally administered budesonide-loaded PLA nanoparticles (345 nm) in Sprague-Sawley rats. The effect of the system was compared with a single injection of a budesonide solution, and drug levels in retina and other tissues were determined at 1, 3, 7 and 14 days post-injection. At the end of day 7, budesonide concentration in cornea and vitreous was higher in the group treated

with nanoparticles than in controls. Although on day 14, drug levels were below limits for both solution and budesonide NPs groups in all the tissues analysed [104].

Kalita et al. carried out a preliminary study to examine the safety of a single posterior sub-tenon injection of 1 mL (10 mg/mL) of carboplatin-loaded polymethylmethacrilate nanoparticles prepared by free-radical emulsion polymerization of methyl methacrylate from an aqueous solution containing carboplatin (110±10 nm, polydispersity index 0.2, negatively charged) for the treatment of advanced retinoblastoma in humans [116]. . Six patients were randomly distributed to receive a fixed dose of 10 mg/mL posterior sub-tenon injection of freshly prepared nanoparticle suspension at 6 hours, 24 hours or 72 hours prior to enucleation. The level of carboplatin was analyzed in ocular tissues and eyes were evaluated for evidence of choroidal and retinal toxic effects. Authors concluded that nanoparticulate forms of carboplatin may provide facilitated delivery across sclera to the intraocular target, without associated short-term tissue and systemic side effects in humans. However, they claimed that a larger clinical trial in humans was needed to assess clinical efficacy and long-term toxicity.

Feng et al. evaluated the ocular distribution of alexa647-labeled pRNA nanoparticles (pRNA-3WJ and pRNA-X) after subconjunctival injection in female mice and their feasibility to deliver the nanoparticles (8-12 nm) to corneal and retinal cells. They performed imagine studies in the whole-body and dissected ocular tissues, as well as study the distribution and clearance of the nanoparticles by monitoring the eyes *in vivo*. Both pRNA-X and p-RNA3WJ were found in conjunctiva, cornea and sclera cells after the injection. However, only pRNA-X was found in retina. Authors suggested that this situation was probably related to the sizes and shapes of the nanoparticles. They also noticed an important clearance of the systems through the cervical lymph node.

Liposomes

Interestingly, liposomal nanocarriers have been used as novel systems of vaccine delivery at periocular level. Cortesi et al. have demonstrated the main role of periocular vaccination for preventing HSV infection in rabbits [117-119]. In these studies, authors developed liposomes composed by PC:Cht: Dioctadecyl-dimethyl-ammonium bromide and a recombinant secreted form HSV-1 glycoprotein B(gB1s) with a protective immunity activity and two peptides mimicking gB1s, DTK1 and DTK2, with additional antiherpetic activity (300 nm; +23 mV). Rabbits were given a subconjunctival vaccination three times at 3-week intervals. Then, they were infected with pathogenic HSV-1 variant and clinical eye signs and symptoms (conjunctivitis, iritis, epithelial keratitis, and corneal clouding) were evaluated on day 3, 5, 7, 10 and 14 post-infection.

Six out of nine animals treated survived in comparison with controls (animals vaccinated with unloaded liposomes). These results support the concept that local periocular vaccination can be potentially used as a successful therapeutic vaccine against recurrent HSV-1 ocular shedding [120]. Elsaid et al. prepared and characterized sirolimus-loaded nanocarriers using cholesterol-poly(ethylene) glycol polymers (Cht-PEG, 1,000 or 5,000 g/mol) to assess their effect on transcleral permeation and drug retention [121]. Particle size ranged from 11.7 to 16.2 nm with a narrow particle size distribution. The permeability and diffusion coefficients did not vary significantly between Cht-PEG polymers, being 14-fold lower than the drug dissolved in organic solvents such as dimethyl sulfoxide or methanol, known to enhance ocular permeation. The sclera drug retention was 13-28 folds higher than that of the diluted sirolimus solution. Formulations showed a good stability, with little change in the size of the nanocarriers over time, and a good drug loading and entrapment efficiency (77-82%). Negatively-charged Cht-PEG 5,000 g/mol had lower sclera drug retention than the neutral Cht-PEG 1,000 g/mol, although both showed successful transcleral drug retention and permeation. According to authors, results suggested that Cht-PEG nanocarriers are potential tools for the delivery of lipophilic drugs to the anterior or posterior segment of the eye.

Dendrimers

Dendrimers have been also explored in the peroicular route. Shaunak et al. conjugated PAMAM dendrimers and glucosamine or glucosamine 6-sulfate to obtain immuno-modulator systems or antiangiogenic systems respectively. The conjugates were subconjunctivaly co-administered in a rabbit model of wound healing after glaucoma filtration surgery showing an increment of the long-term success of the surgery from 30% to 80%, and also a reduction in both inflammation and angiogenic responses. Furthermore, the protection was observed 30 days post injection [122].

Kang et al. [123] prepared nanoparticles (258 nm) by aggregation of host-guest PAMAM-carboplatin systems. These nanoparticles were administered in retinoblastoma animal models (LHβ-Tag mice) by subconjunctival injection. The mean tumour mass in eyes was significantly reduced after injection of the dendrimeric systems in comparison to the non-treated group and also in comparison to the group treated with carboplastin aqueous solution due to the prolonged retention of nanoparticles and the sustained drug delivery.

POTENTIAL CLINICAL TRANSLATION

In spite of the clear advantages of using nanoparticles, their use for topical administration has some limitations, derived from the tolerance and the penetration ability of the particles across the ocular tissues. They provide beneficial effects such as enhancement of drug bioavailability. However, a repeated administration can cause accumulation of particles resulting in a damage of the tissue if the rate of the nanosystem biodegradation is lower than the interval dosing. Furthermore, special attention must be paid in the tolerability of the formulation, mainly if they are destined to treat chronic diseases. In fact, there are interesting nanosystems under the technological point of view with low possibilities to reach the clinic because of their associated toxicity. For this reason, it is very important to assure the whole elimination as well as a high tolerance of the nanosized formulations. This can be done by the use of non-toxic biodegradable materials.

Hybrid formulations composed by nanoparticles, liposomes or niosomes combined with polymeric solutions have been explored with good results in terms of tolerance and efficacy. Furthermore, the increase of the viscosity of the formulations thanks to the use of these polymeric solutions would improve the suspension stability. Polymers employed in the clinical practice, for the treatment of ocular surface alterations combined with nanovesicles, resulted especially interesting in long-term treatments and could contribute to patient adherence to treatment.

The use of positively charged microemulsions and also the inclusion of bioadhesive polymers in the aqueous phase of the emulsion have shown promising results. However, it is important to note that positively charged formulations resulted usually in lower values of tolerance than the ones observed for negative and non-charged microemulsions.

Nanoparticles, liposomes and dendrimers present great interest in gene therapy and to protect the active substance improving the therapeutic effect. For the back of the eye, the diffusion of the nanosized systems throughout the vitreous to the target cells after their injection is a critical issue being under investigation.

Biodegradable microparticles are emerging tools to treat chronic diseases affecting the back of the eye. These novel formulations are able to release the active substance for long periods of time compared to nanoparticles. Also, microsized particles can contain different active substances (multi-loaded systems) in the same device resulting useful in the treatment of multifactorial diseases. It is important to assure that particles suffer

aggregation after being injected, avoiding the interference with vision. The aggregation phenomena would promote a slow delivery of the drug extending the therapeutic effect although this fact has to be controlled. Tolerance of microparticles intended for the treatment of posterior segment diseases is a critical issue as the retina and surrounding tissues resulted fundamental in vision. Similarly to topical administration the polymers employed must present good tolerance with the intraocular tissues.

The combination of nano- and microsystems in the named “Trojan” devices results also a very interesting tool for the treatment of diseases in the posterior segment of the eye so they combined the benefit of both systems.

In summary, the right choice of the ophthalmic drug delivery system depends on the ophthalmic disease, the ocular target site and the route of administration.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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CAPTIONS

Fig 1: Eye structure and different local administration routes intended for the treatment of ocular diseases.

Fig 2: Structure of the retina. The main neurons forming the retina are schematically presented: retinal ganglion cells (RGCs), amacrin cells, bipolar cells, horizontal cells and photoreceptors (cones and rods). The retinal pigmented epithelium (RPE) is part of the blood retinal barrier and controls exchange of nutrients with the choroidal vessels.

Fig 3: Structure of the precorneal film. The lipid layer, composed mainly by phospholipids, covering the aqueous layer, avoids water evaporation. Ions, enzymes, etc. and mucins (soluble and gel-forming) are dissolved in the aqueous layer. The transmembrane ocular mucin layer connects the precorneal film with the corneal epithelium.

Fig 4: Structure of the cornea. The stratified corneal epithelium is followed by a predominantly aqueous stroma. The monolayer corneal endothelium is in contact with the aqueous humour. The main barrier for active substances entrance is the stratified epithelium that can be crossed by intracellular or paracellular pathway.

Fig 5: Structure of micro and nanoparticles. While in micro and nanospheres the active substance is in intimate contact with the polymer forming a matrix system, in the micro and nanocapsules it is confined in a reservoir core surrounded by the polymer.

Fig 6: Structure of liposomes. Hydrophobic active substances can be loaded in the apolar lipid bilayer while hydrophilic active substances can be included inside the vesicle or adsorbed on the surface.

Fig 7: Structure of microemulsions and Niosomes. Micoremulsions can load hydrophobic active substances in the O-phase. In niosomes the active substance can be located in the surfactant bilayer (hydrophobic compounds) or inside the vesicle or adsorbed on the surface (hydrophilic compounds).

Fig 8: Structure of dendrimers. These hyper-branched polymers can interact with active substances inside their structure (mainly by hydrophobic interactions) or on their surface (both by covalent and non-covalent bindings)

Figure

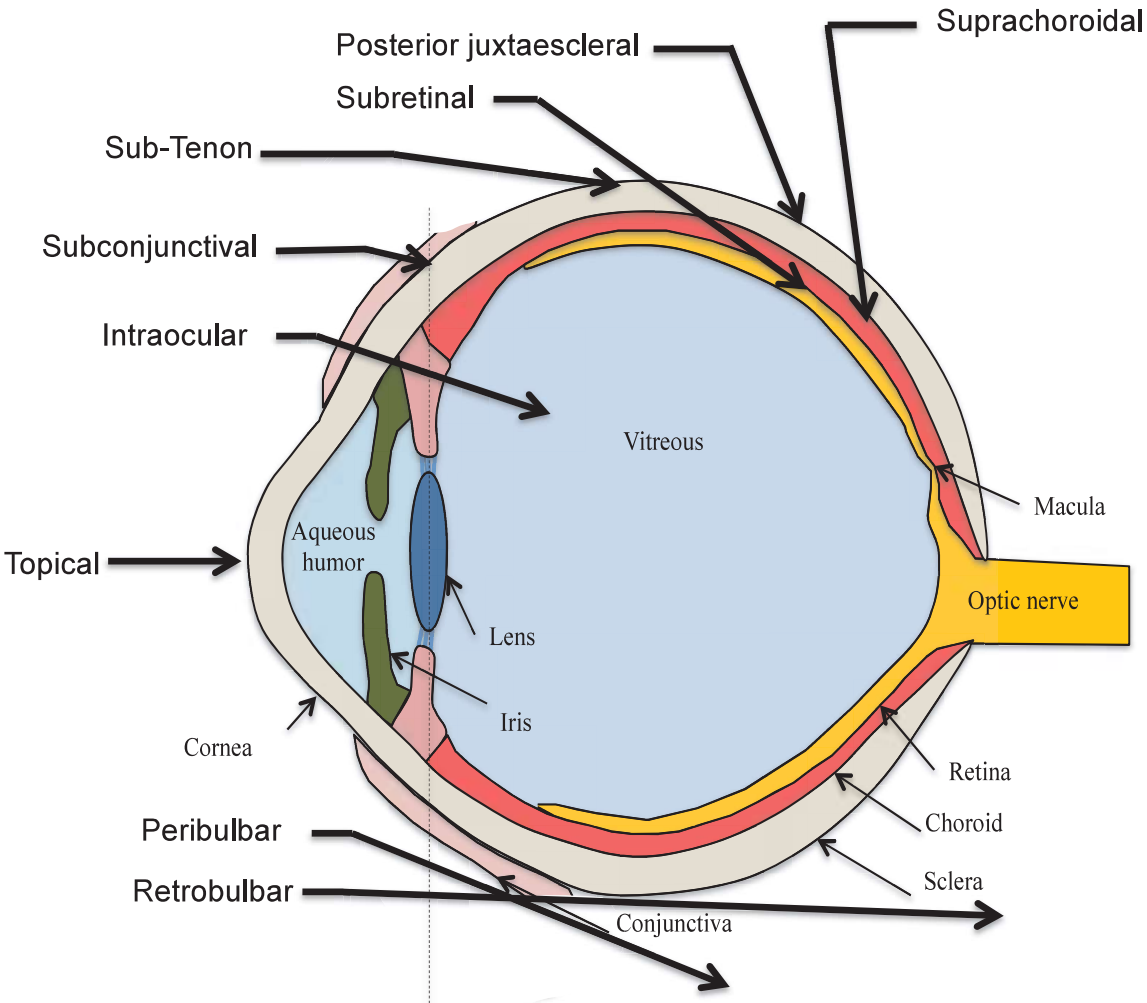

Fig.1 Anterior segment Posterior segment

Figure

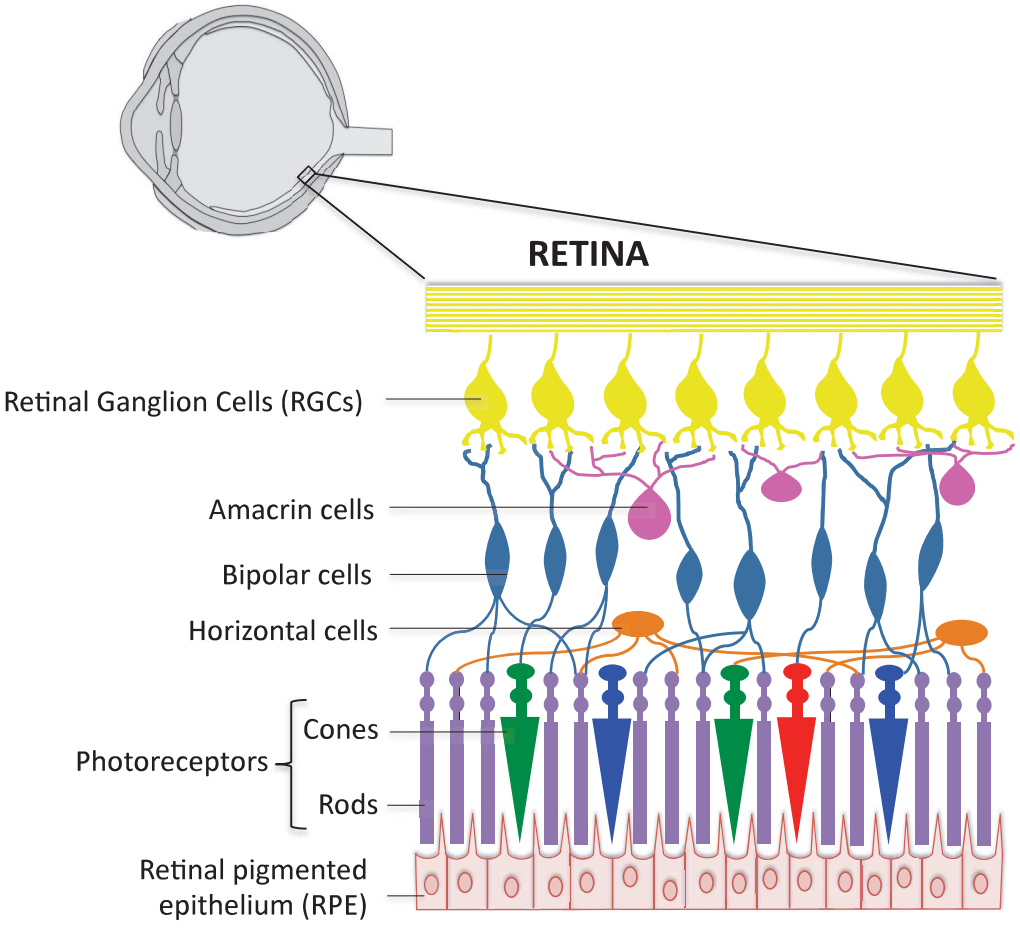
RETINA
Retinal Ganglion Cells (RGCs)
Amacrin cells
Bipolar cells
Horizontal cells
Photoreceptors
Cones
Rods
Retinal pigmented epithelium (RPE)

Fig.2

Figure

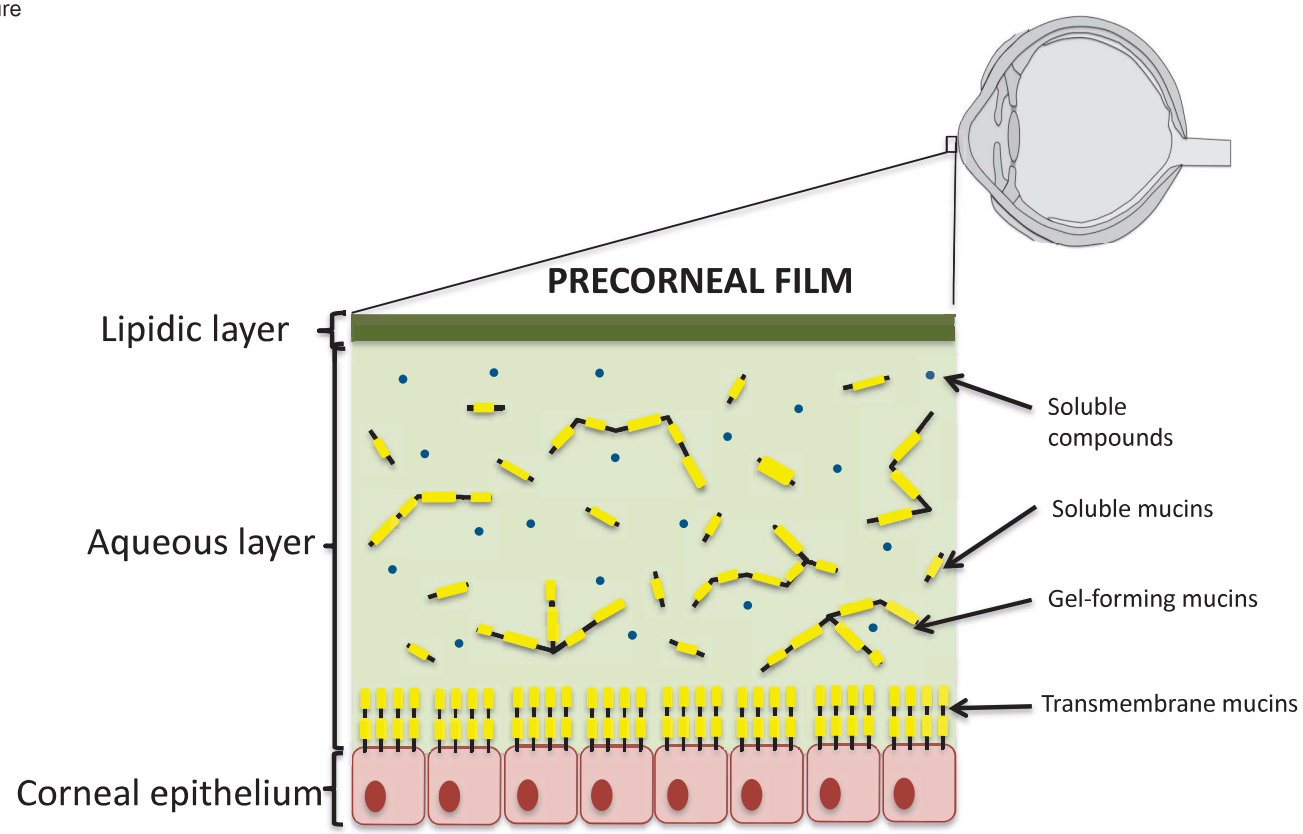
PRECORNEAL FILM
Lipidic layer
Aqueous layer
Corneal epithelium
Soluble compounds
Soluble mucins
Gel-forming mucins
Transmembrane mucins

Fig.3

Figure

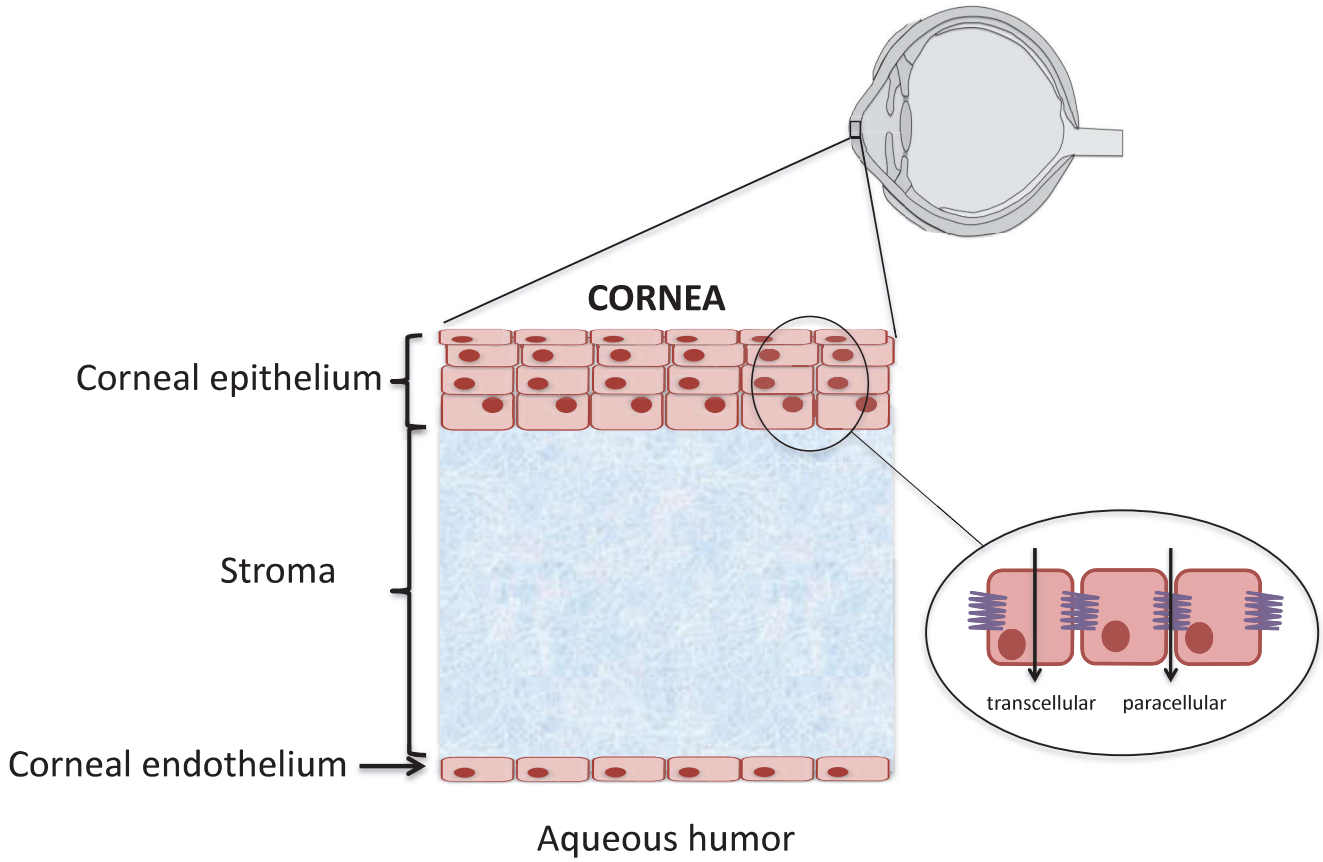
CORNEA
Corneal epithelium
Stroma
Corneal endothelium
transcellular
paracellular
Aqueous humor

Fig.4

Figure

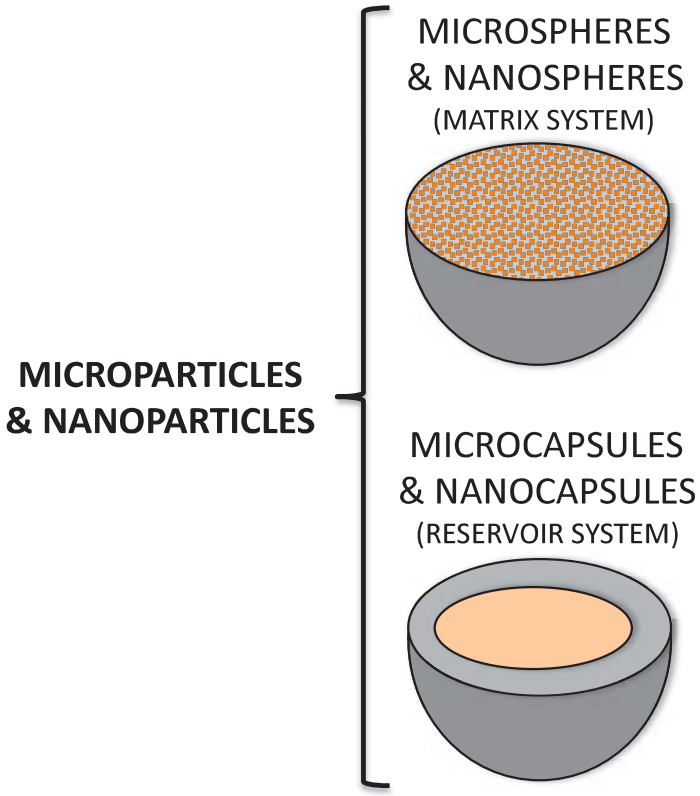
MICROSPHERES
& NANOSPHERES
(MATRIX SYSTEM)
MICROPARTICLES
& NANOPARTICLES
MICROCAPSULES
& NANOCAPSULES
(RESERVOIR SYSTEM)

Fig.5

Figure

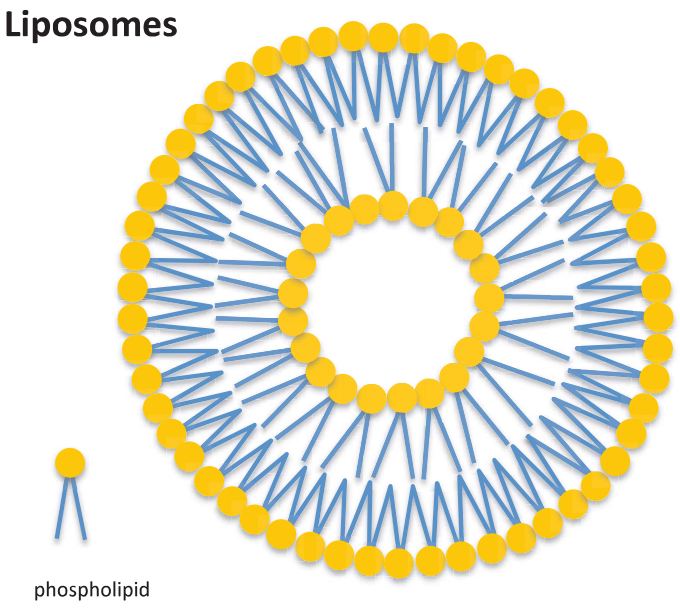

Fig.6

Figure

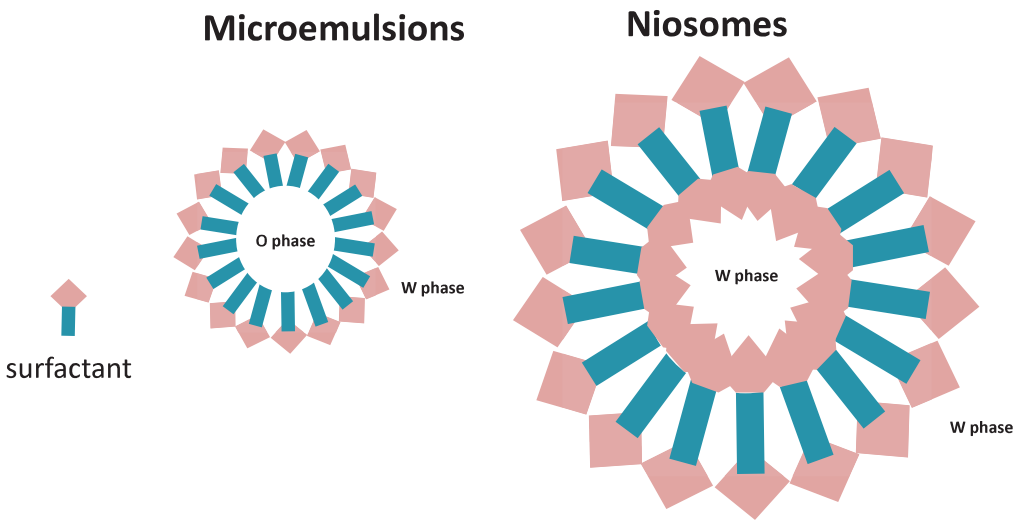
Microemulsions
Niosomes
O phase
W phase
surfactant
W phase
W phase

Fig.7

Figure

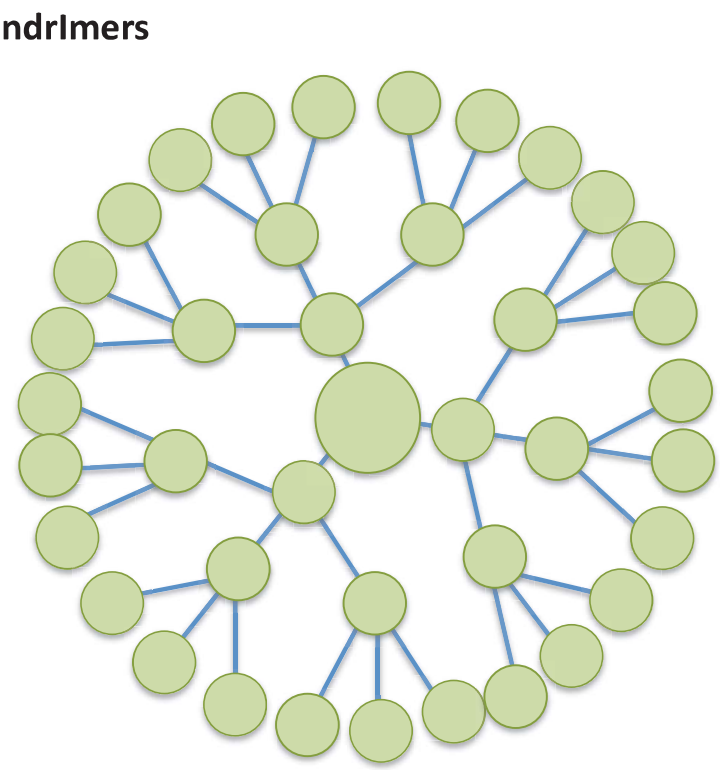

Fig.8