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Cyclosporine A delivery to the eye: a comprehensive review of academic and industrial efforts

Frédéric Lallemand[a], Mathieu Schmitt[a], Jean-Louis Bourges[b], Robert Gurny[c], Simon Benita[d], Jean-Sébastien Garrigue[a]

[a]Santen SAS, Evry, France
[b]Department of Ophthalmology, Paris Descartes School of Medicine, Assistance Publique-Hôpitaux de Paris, Cochin-Hôtel-Dieu Hospital, Université Sorbonne Paris Cité, Paris, France
[c]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland
[d]The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Ein Kerem, Israel

Corresponding Author

Name: Jean-Sébastien Garrigue, PharmD, MBA

Affiliation: Santen SAS, 1 rue Pierre Fontaine, 91000 Evry, France

Email: jean-sebastien.garrigue@santen.fr

Phone: 33 1 69 87 40 20

Fax: 33 1 69 87 40 30

Abstract (100-200 words)

Local ocular delivery of cyclosporine A (CsA) is the preferred method for CsA delivery as a treatment for ocular inflammatory diseases such as uveitis, corneal healing, vernal keratoconjunctivitis and dry eye disease. However, due to the large molecular weight and hydrophobic nature of CsA and the natural protective mechanisms of the eye, achieving therapeutic levels of CsA in ocular tissues can be difficult. This review gives a comprehensive overview of the current products available to clinicians as well as emerging drug delivery solutions that have been developed at both the academic and industry levels.

Keywords (5-10 max)

Ocular delivery; Cyclosporine; Solution; Emulsion; Hydrogel, Pipeline products

Abbreviations

Aq-CsA: CsA in a micellar solution, AUC: area under the curve, BAK: benzalkonium chloride, bid: twice daily, CKC: cetalkonium chloride, CMC: critical micellar concentration, CsA: cyclosporine A, DDS: drug delivery system, DED: dry eye disease, EDTA: ethylenediaminetetraacetic acid, Em-CsA: CsA in an oil-in-water emulsion, EU: European Union, EVEIT: *Ex Vivo* Eye Irritation Test, HA: hyaluronic acid, HEMA: 2-hydroxyethyl methacrylate, ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, KCS: keratoconjunctivitis sicca, MCT: medium-chain triglyceride, MD: multi-dose container, mPEG-hexPLA: methoxy poly(ethylene)glycol-hexyl substituted poly(lactides), NA, not available; NaCl: sodium chloride, NaOH: sodium hydroxide, Oil-CsA: CsA in a castor oil solution, PLGC: poly(lactide-co-glycolide-co-caprolactone), p-HEMA: poly-HEMA, PLGA: poly(lactic-co-glycolic acid), qd: once daily, SCF-CO_2: supercritical fluid of carbon dioxide, SFA: semifluorinated alkane, TBC: to be confirmed; TFLL: tear film lipid layer, tid: three times daily, TJ: Taejoon; UD: unit-dose container, US: United States, VKC: vernal keratoconjunctivitis, WW: worldwide

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1. Introduction

Cyclosporine A (CsA) is a cyclic undecapeptide of 1202.6 Da that was discovered in the 1970s by Sandoz [1]. Given its strong immunosuppressive potency, it was first developed to counter graft rejection following organ transplantation [2]. Its large molecular weight and hydrophobic nature (Log P=1.4 to 3.0; solvent dependent) are responsible for its low aqueous solubility (6.6 to 106 µg/mL; temperature dependent) [3, 4], necessitating the development of drug delivery strategies to maximize its bioavailability. CsA was initially marketed as an injectable ethanolic solution in the mid-1980s and then as an oily solution from the early 1990s, under the trade name Sandimmune® [5]. In the mid-1990s, the formulation was modified to improve its systemic bioavailability. Since then, CsA has been developed as a self-microemulsifying drug delivery system marketed under the trade name Neoral® as an oral solution and as soft gel capsules. In liver-transplanted patients, CsA area under the curve (AUC) and maximum serum concentration (C_{max}) were 50% and 90% higher, respectively, following Neoral® treatment compared with Sandimmune® treatment [6].

In ophthalmology, CsA use was investigated as early as 1981, initially for administration after corneal graft transplantation [7]. CsA also showed interesting activity in uveitis, corneal healing, vernal keratoconjunctivitis and other inflammatory diseases of the eye [4, 8]. Initially, CsA was administered orally for the treatment of ophthalmic diseases. Although CsA was able to reach therapeutic levels in different ocular tissues [9], the non-ocular administration led to occurrence of systemic adverse events such as nephrotoxicity, hypertension, anemia, paresthesia and hyperesthesia [10]. Therefore, local (ocular) administration of CsA-loaded products became the preferred method of delivery for treatment of ophthalmic pathologies.

Given that the eye is essentially an extension of the central nervous system, ocular drug delivery is particularly challenging, especially for the local delivery of poorly water-soluble and/or large molecules. The eye possesses a multitude of protective mechanisms against external threats such as dust, xenobiotics and pathogens. These defense mechanisms (e.g. blinking, tearing and tear film turnover) are extremely efficient in clearing foreign elements from the ocular surface [11]. However, they also lead to very poor drug penetration for topically applied ocular drugs, typically below 5% [12]. Over the past two decades, several drug delivery strategies have been investigated that enhance the ocular bioavailability of CsA following topical instillation to achieve and improve disease management without the CsA-induced systemic adverse effects associated with oral administration (Figure 1). These efforts finally led to the

commercialization of several eye drops based on various drug delivery systems. The first ophthalmic product on the United States (US) market was Restasis®, approved in 2002 [13] and launched in 2003 by Allergan, whereas Santen (Novagali) became the first company to reach the European Union (EU) market with CsA eye drops (Ikervis®) in 2015 [14]. Both products utilize nanoemulsion drug delivery systems and are intended for the treatment of dry eye disease (DED).

In 2003, Lallemand *et al.* [4] published a comprehensive review on CsA ocular delivery. At that time, Restasis® was the only commercially available CsA ophthalmology product approved for use, and it was licensed only in the US, not in the EU. Presently, the topical ocular CsA landscape has evolved substantially, with newly available marketed products and new companies developing innovative CsA-based products for ophthalmic diseases. This review gives a compendium of the ophthalmic CsA products that are currently available as well as emerging drug delivery systems that are being developed to treat dry eye disease in academic as well as industry settings.

2. Available products

The use of CsA in DED has been described for several decades [15], yet only a few products have been successful in reaching the pharmaceutical market place (Table 1). The main ocular indications that these marketed CsA products are used for include DED and severe allergy (e.g. vernal keratoconjunctivitis, VKC). Alternately, hospital-compounded formulations have been widely in use for a range of diseases of the ocular surface and anterior segment [16-18].

Because of the very poor aqueous solubility of CsA, formulation strategies have focused on developing novel mechanisms to deliver a solubilized state of the drug to the corneal surface. Additional challenges in ophthalmic formulations include safety and tolerability of the product for direct ocular application. These eye drop products should be devoid of any inactive excipients that have unwanted ocular surface altering properties (e.g. penetration enhancers like laurocapram, which is cytotoxic to the corneal epithelium, and Cremophor®, which is associated with structural changes to the corneal surface) [4, 19]. Ideally, these formulations ought to be preservative free and should replenish and/or stabilize the tear film layers without disturbing the ocular surface system homeostasis and functions.

As a consequence of these limitations and challenges, oil-in-water emulsions (e.g. Restasis®, Lacrinmune® and Ikervis®) and micelle-based solutions (e.g. Papilock Mini®, Modusik-A Ofteno®

and Taejoon [TJ] Cyporin®) have been successfully developed and registered to treat DED (Table 2) [20-23]. In addition, Schering-Plough produces and markets a CsA ophthalmic ointment for the treatment of keratoconjunctivitis sicca (KCS) in dogs [24]. All of these ophthalmic products significantly lubricate the eye because they are mostly (>90%) composed of water [12]. The oily excipients additionally benefit the ocular surface by restoring the lipid layer of the tear film and protecting the aqueous layer from drying out [25, 26]. Interestingly, none of these products contain the most frequently used ophthalmic preservative agent in glaucoma drugs, benzalkonium chloride (BAK), which is reported to be deleterious to the corneal tissues [27]. Unique among the marketed products, the Ikervis® formulation contains quaternary ammonium cetalkonium chloride (CKC), which acts as an emulsifier as well as a cationic surfactant to stabilize the colloidal system. It was demonstrated that CKC in Ikervis® neither has a preservative role nor exhibits any corneal toxicity [8, 26]. It additionally exhibits an ocular safety profile similar to that of Restasis® and hospital-compounded CsA formulations [8]. A study using human corneal cells showed that a CKC-based nanoemulsion had similar effects on cell survival to a phosphate-buffered saline solution [28].

It is worth noting that none of the currently licensed products are globally available. This may be because some of these products were developed by local companies and launched only within their country or a specific region (i.e. Modusik-A Ofteno® by Sophia Laboratorios in Mexico and Latin America or TJ Cyporin® by Taejoon Pharm in South Korea) [23]. Alternatively, a product may have been developed for potential global use but failed to receive marketing authorization across all regions (e.g. Restasis® was approved by the US Food and Drug Administration [13] but is not currently approved for use in all EU countries [14]). This discrepancy reflects differences in regulatory guidelines and the interpretation of clinical trial results from one country/region to another.

2.1. Solutions

Aqueous solutions are usually the simplest eye drop formulations, as their composition and manufacturing processes are relatively simple. Aside from their ease of preparation, a potential advantage of solutions is enhanced bioavailability of the drug substance given that it is already dispersed at the molecular level. However, a significant concern with ocular solutions is the dilution and consequent washing away of the solvent with tear fluid, leaving behind a solute precipitate that could limit bioavailability of the drug to the tissues located in the front of the eye. For CsA, which is poorly soluble in water, surfactants and co-solvents (e.g. ethanol) are required to form a solution that remains stable throughout its shelf life [25]. The addition of

surfactants to the solution helps in the formation of small micelles, which arise as the surfactant molecules aggregate with their hydrophilic portions orientated outwards towards the water and their hydrophobic parts clustering inwards, encapsulating the lipid-soluble CsA [29]. Surfactants form micelles when present in water at concentrations above the critical micellar concentration (CMC). Bioavailability of the CsA is enhanced by the solubilizing effect of micelle formation and also by the high permeation through biological membranes that the micelles provide [29], being typically 20 to 50 nm. Once instilled in the eye, the concentration gradient facilitates absorption of the drug by adjacent tissues. In addition, the large specific surface area of micelles increases the bioavailability of the drug [29]. Sometimes, tear fluid dilution of the eye drop could reduce the surfactant concentration below its CMC, thereby leading to the precipitation of CsA. However, this phenomenon occurs relatively slowly due to the low dilution ratio of the tear film, thus providing ample time for drug absorption.

High concentrations of surfactant are deleterious to biological tissues, and surfactants are not specific in terms of enhancing permeation. A potential disadvantage of aqueous solutions is their long-term tolerability, since repeated instillations of surfactant-containing formulations could potentially affect the ocular surface tissues [30]. To avoid the abrasive effects of ionic emulsifying agents, the use of non-ionic surfactants is preferred for aqueous solution formulations.

Since 2003, several CsA solutions have been developed and successfully marketed. In these products, CsA is dissolved with the help of ethanol and non-ionic surfactants, such as polysorbate 80 and polyoxyl-40 stearate. Papilock Mini® (Santen), approved for use in Japan since 2005, is a 0.1% CsA aqueous solution administered three times per day for the treatment of vernal keratoconjunctivitis (VKC) [31]. This formulation of Papilock Mini® VKC includes hydroxyproplyl methylcellulose as a viscosity-modulating agent, which enables the solution to remain on the ocular surface by physical means of retention, consequently increasing the residence time of the product [31-33].

In Mexico and several other Latin American countries, Modusik-A Ofteno®, commercialized by Sophia Laboratories, is indicated for the treatment of DED [21]. The formulation is based on the patented Sophisen™ platform (ethanol and a combination of surfactants) and designed to improve the solubilization and corneal bioavailability of poorly water-soluble drugs.

TJ Cyporin N® (Taejoon Pharma) and Cyporin® (Aristopharma Ltd) are aqueous solutions containing 0.05% w/w CsA and are available in some countries in Asia, including South Korea, Bangladesh and Myanmar [23].

2.2. Emulsions

As CsA is highly soluble in various oils, e.g. medium-chain triglycerides (MCTs), castor oil and olive oil, lipid-based formulations are particularly well suited for CsA delivery [4]. The use of lipid-based emulsions is a strategy that has notably been used by Novartis (formerly Sandoz) for the development of Sandimmune® and Neoral®, which are classified as self-emulsifying drug delivery systems [34]. These formulations are isotropic mixtures of oils, surfactants, solvents and co-solvents/surfactants, and they are particularly attractive for an oral product since the (micro) emulsion gets formed spontaneously *in vivo* upon dilution of the lipid-based formulation in gastrointestinal fluids. However, this strategy is inappropriate for corneal instillation because the large amounts of surfactants could have deleterious effects on the cornea and could result in blurred vision. Hence, conventional oil-in-water emulsions have been developed to deliver CsA to ocular tissues located in the front of the eye. These formulations have a number of advantages: they spread readily over the ocular surface, maximize the specific surface given the nano-sized oil droplets and are well tolerated (low surfactant quantity [12, 26]). In addition, because the drug is already dispersed at the molecular level and encapsulated within the oil droplet, the risk of precipitation on the ocular surface is avoided. Similar to these emulsions, the tear film itself is comprised of a lipid and an aqueous phase [35]. Therefore, it is possible that a fraction of the oil droplet encapsulating CsA may merge with the tear film lipid layer (TFLL), entrapping a portion of active drug within. Since the TFLL turnover is much longer than that of the aqueous phase, TFLL may act as a drug reservoir and allow for sustained release of CsA [36]; however, there is no direct evidence to confirm this hypothesis thus far.

Although nanoemulsions are more complex formulations than conventional aqueous eye drop solutions, they can be easily manufactured on a large scale using specific equipment, such as high-pressure homogenizers, and simply sterilized by filtration or autoclave. In 2003, Allergan was the first company to bring a licensed formulation of CsA eye drops to market. Restasis®, a preservative-free anionic oil-in-water nanoemulsion, contains CsA dissolved in castor oil with polysorbate 80 as the emulsifying agent. The resulting emulsion is further stabilized by carbomer copolymer [22]. The marketing authorization for Restasis® in the United States was granted by the FDA in 2002 [13]. However, Restasis® is presently not approved in the EU, and its use is only allowed for compassionate use [14].

Bausch & Lomb launched Lacrinmune® in Argentina, which has a composition similar to that of Restasis®, except for the addition of sodium hyaluronate to the emulsion [37]. Hyaluronic acid derivatives are well known and are widely used in the management of mild to moderate dry eye syndrome, for example Vismed® (TRB Chemedica), Hyalein® (Santen) and Opticalmax® (Omega Pharma). The addition of sodium hyaluronate increases the viscosity of the emulsion, resulting in a longer residence time on the ocular surface than that of aqueous eye drops.

In 2015, Ikervis® (Santen) was granted marketing authorization by the European Medicines Agency for use in Europe. Ikervis® is a cationic nanoemulsion indicated for the treatment of severe keratitis in adult patients with DED that has not improved despite treatment with tear substitutes [38]. The cationic nanoemulsion is a patented technology based on the Novasorb® platform developed by Novagali Pharma, France (now Santen SAS). Due to the net positive charge of the oil nanodroplets, the residence time and the ocular bioavailability of CsA are higher with the cationic emulsion than with other formulations. In a pharmacokinetic study on rabbit eyes, corneal exposure to CsA after a single dose was 1.84 times greater for a 0.05% CsA cationic emulsion (Novasorb® formulation) than for a 0.05% CsA anionic emulsion (Restasis®); $AUC_{0–72\ h}$ 26,703.0 ng·h/g and 14,333.2 ng·h/g, respectively [39]. Correspondingly, the corneal clearance of CsA was 57% less for the 0.05% CsA cationic emulsion than for the 0.05% CsA anionic emulsion (0.8 g/h and 1.4 g/h, respectively). It is assumed that the residence time of CsA in Ikervis® (Novasorb® CsA cationic nanoemulsion) is greater than that in Restasis® (CsA anionic nanoemulsion) because of electrostatic interactions between the positively charged droplets and negatively charged mucus protein of the corneal epithelium [12]. This mechanism of action would work in conjunction with the hypothesized reservoir effect of the TFLL. The combination of these effects, as well as higher dosage strength, could very likely explain the difference in dosing regimen between once-a-day Ikervis® versus twice-a-day Restasis®.

Kuwano *et al*. [40] compared the ocular pharmacokinetics of three CsA formulations in rabbit eyes: a castor oil solution (Oil-CsA), a micellar solution (Aq-CsA) having a composition similar to Papilock mini® and an oil-in-water emulsion (Em-CsA) with a composition similar to Restasis®. This study reported that the $AUC_{0–12}$ of Em-CsA and Aq-CsA were, respectively, 9.2- and 28.5-fold higher than the $AUC_{0–12}$ of Oil-CsA in corneal stroma endothelium. The same pattern was observed in the bulbar conjunctiva, wherein the $AUC_{0–12}$ of Em-CsA and Aq-CsA were 2.4-fold and 5.1-fold higher than the AUC_{0-12} of Oil-CsA, respectively [40]. These results clearly showed that micellar solutions of CsA improve the ocular bioavailability of CsA. Improving the

bioavailability of CsA in ocular tissues is obviously required to maximize the clinical efficacy of CsA products. Nevertheless, DED is a very complex disease with diverse signs and symptoms, and an optimal treatment may require additional interventions alongside pharmacological therapy to achieve optimal outcomes.

2.3. Others

Besides ophthalmologic use in humans, CsA has been available for veterinary applications since the late 1980s [41, 42]. Optimmune® (Merck), a 0.2% CsA ophthalmic ointment of petrolatum, corn oil and lanolin alcohol, has been commercialized by Intervet for treatment of KCS and superficial keratitis in dogs [24]. Theoretically, residence time of ointment formulations on the ocular surface tissues is longer than that of eye drops [43]. In addition, administration of this semi-solid formulation may be easier than liquid drops in dogs.

3. Hospital-compounded preparations

As the availability of marketed ophthalmic products containing CsA is limited, especially for higher strengths (0.5–2% w/w), compounded formulations are prepared in hospital pharmacies. A retrospective study examining of compounded CsA eye drops (oil solution) prepared by the Hôtel-Dieu de Paris Hospital (Paris, France) noted that 2% CsA solution is the most commonly prepared formulation (44,243 preparations out of a total of 62,635 in 2013), and this dosage strength was primarily used for the prevention of corneal graft rejection (61% of cases) [44]. Lower dosage strengths (0.5% and 0.05%) were typically used to treat dry eye syndrome, Gougerot-Sjögren syndrome, ocular rosacea and severe allergies (atopic keratoconjunctivitis and VKC), as well as to prevent high-risk corneal graft rejection. The compounded preparations of CsA for ophthalmic use vary widely in composition and dosage strengths as inferred from a recent pan-European survey of ophthalmologists on the use of ophthalmic hospital preparations of CsA [18]. While the survey indicated that most of the compounded formulations of CsA consisted of a mixture of Sandimmune® solution (either oral or injectable) or Optimmune® (0.2% veterinary ointment) and artificial tears or oils, it also demonstrated the differences in CsA formulations that are dispensed for treating specific ocular conditions across the hospitals surveyed.

Knagenhjelm *et al.* [45] demonstrated the absence of ocular toxicity in formulations of CsA (Sandimmune® oral solution or Sandimmune® infusion concentrate) mixed with peanut oil through a short-term study on rabbit eyes. A study by Benitez del Castillo *et al.* [46] showed no significant differences in the increase in permeability of 2% CsA in olive oil (7.03 times) versus

olive oil alone (6.68 times) into the corneal epithelium after a single instillation of the compounded preparation (*P*=0.651). The authors of this study concluded that olive oil, by itself, increases corneal permeability through a toxic effect on the corneal epithelium and recommended reducing the use of olive oil solutions of CsA to avoid the potential side-effects. Nevertheless, given the heterogeneity in the composition and the operating processes for preparing and packaging of drugs among compounding pharmacies, significant differences in the efficacy and tolerance between these CsA formulations are inevitable.

Other compounded CsA formulations have also been reported in literature. Bontemps *et al*. [47] describe formulation of 0.1%, 1% and 2% CsA eye drops by evaporating the ethanol contained in Sandimmune® injection solution and replacing this volume with artificial tears (Dulcilarmes®). Although the CsA content of the resultant solution was stable when stored for 90 days in the refrigerator, the relative insolubility of CsA in water increases the risk of crystallization of CsA with time. Furthermore, the replacement of ethanol with Dulcilarmes® results in a 0.1% CsA solution with an approximate osmolality of 430 mOsmol/kg that is significantly more hypertonic than the tear fluid (mean value 303.7 mOsmol/kg) and is potentially a trigger for ocular surface inflammation [48]. In contrast, Chast *et al*. [49] developed an oily solution with one part of Sandimmune® oral solution diluted in four parts of castor oil, both constituents filtered under laminar flow using 0.22-µm filters to ensure sterility of the final preparation. This oily injection solution contained less ethanol from the Sandimmune® solution and was stable at room temperature even after 12 months of storage, preserving nearly 98.5% of the CsA content as the theoretical initial concentration. Nonetheless, the packaging vials were produced as multi-dose containers, necessitating monitoring of the sterility of the product throughout the period in use. There were no adverse events observed in the 250 patients in whom this product was used for the prevention of graft rejection or in immune disease of corneal tissue, demonstrating the tolerability and suitability of this vehicle for use as eye drops [49]. The long-term stability and sterility of CsA was further optimized for daily use by patients by packaging in an easy to handle, low-density polyethylene container [50].

Table 3 summarizes the differences between hospital-compounded preparations of CsA eye drops that could impact the tolerance and efficacy of the product. If Sandimmune® (oral and intravenous formulations) were not available, pharmacies would need to resort to handling CsA raw material, which requires specific protective equipment to guarantee the safety of operators because of its CMR (carcinogenic, mutagenic or toxic for reproduction) classification [51-53]. Moreover, every preparation of the product would need to be validated to ensure shelf-life

stability. Therefore, access to ready-to-use CsA products consistent with large-scale production according to Good Manufacturing Practices (GMP) would complement the supply of hospital-compounded preparations to the advantage of both patients and ophthalmologists.

4. Products in commercial development

4.1. MC2BIOTEK (PADciclo™)

PADciclo™, being developed by the Danish biotech company MC2BIOTEK, comprises a dispersion of polyaphrons encapsulating CsA within oily micrometer-sized aphrons dispersed in a hydrogel of carbomer [54, 55]. Polyaphrons are lipid-based formulations first described in the late 1970s [56]. The main advantage of polyaphrons over an emulsion is the high concentration of the dispersed oil phase that is nearly up to 90% in proprietary formulations [57]. Moreover, the proportion of surfactant required to maintain stability of the dispersed phase is very low, typically less than 0.5%, resulting in a surfactant-to-oil ratio that is much lower than that of emulsions. These attributes make polyaphrons a good vehicle for ocular delivery. However, their cream-like viscosity can make them unsuitable for topical ocular administration. Hence, polyaphrons are more desirable when dispersed in an aqueous vehicle containing a viscosifying agent that not only results in a product with the desired viscosity (i.e. avoids creaming by inhibiting the motion of individual aphrons) but also ensures the long-term physical stability of the product by inhibiting motion of individual globules.

A phase II clinical trial evaluating the safety and efficacy of two dosages of PADciclo™ administered once daily (0.03% and 0.06% w/w CsA) was launched in the summer of 2015 [54]. The CsA concentrations in PADciclo™ are slightly lower than those of existing licensed products (Restasis® 0.05% CsA bid and Ikervis® 0.1% CsA qd), suggesting that the polyaphron technology may improve ocular delivery of CsA. Recent preclinical pharmacokinetic data demonstrated negligible systemic CsA exposure, but after multiple topical PADciclo™ administrations, the conjunctival and corneal penetration of CsA was up to fivefold higher than that achieved with Restasis® [58]. Since 2012, PADciclo™ has been used by 1000+ patients in the UK through a special program at the Moorsfield Eye Hospital [59], although clinical data in these patients has not been made available.

4.2. NOVALIQ (CyclASol®)

Novaliq GmbH is currently developing CyclASol®, a non-aqueous preservative-free solution of CsA formulated using its proprietary EyeSol™ technology. This novel technology uses

semifluorinated alkanes (SFAs) to dissolve poorly water-soluble drugs such as CsA [60]. The drug delivery platform increases the dispersion of CsA and increases the stability of the drug by way of preventing hydrolytic reactions [60]. In addition, CyclASol® has been shown to have greater tissue penetration than Restasis® in an *Ex Vivo* Eye Irritation Test (EVEIT) system. The penetration of the two 0.05% CsA SFA formulations (F4H5 and F6H8) into the anterior chambers of the eye after short-term application was significantly higher (the drug remained for at least 8.5 hours) than that achieved with Restasis® (0.05% CsA), which required repeated instillations for CsA penetration into the aqueous humor [61]. These results suggest that SFA-based formulations may be useful vehicles for delivering drugs into intraocular tissues. In 2014, Novaliq completed a phase I clinical study evaluating the safety and local tolerability of CyclASol® and the systemic exposure to CsA after single and repeated ocular doses (up to four times per day) in 18 healthy volunteers; there were no reports of drug-related signs or symptoms of ocular discomfort, systemic CsA exposure or alterations in the anterior or posterior eye structures [62]. In 2016 Novaliq conducted a phase 2 randomized, double-masked, vehicle-controlled, multi-center US study with four treatment groups, including two CyclASol® groups (0.05% and 0.1%), an open-label active control (Restasis®), and a vehicle control group [63]. 207 patients with moderate to severe dry eye disease were enrolled. Both CyclASol® groups showed a significant improvement in corneal staining compared with the vehicle over the 4-month treatment period. In particular, the central area of the cornea seemed to benefit most, an important aspect for visual function in dry eye patients. All treatment groups demonstrated improvement in symptoms, with CyclASol® showing improvements over vehicle in subgroups. The data further indicated an early onset of action by reduction in corneal and conjunctival staining in as little as 14 days. It is worth noting that the vehicle group also showed significant improvements in symptoms..

Novaliq (through Ursapharm) currently markets EvoTears®, which are also eye drops based on the EyeSol™ SFA technology, designed as a nonblurring wetting lubricant for the ocular surface [64]. The low viscosity and surface tension of this SFA solution results in a much smaller drop size compared with an aqueous solution drop. This property makes an SFA solution suitable for ocular instillation by reducing reflex blinking and blurring.

4.3. APIDEL (ApidSOL™)

Apidel is a Geneva, Switzerland–based drug delivery company currently developing the ApidSOL™ nanocarrier technology [65]. ApidSOL™ nanocarriers are composed of methoxy poly(ethylene glycol) hexyl-substituted poly(lactic acid) polymers that efficiently solubilize

lipophilic drugs. Besides an enhanced solubilization capacity (up to 6 mg/mL; 500-fold of water solubility), ApidSOL™ nanocarriers have a very low CMC (8 mg/L), which suggests high stability [66, 67]. A promising product in Apidel's ophthalmic development pipeline is CsA ApidSOL™, a transparent, colloidal aqueous solution of CsA. Preclinical *in vitro and in vivo* studies have demonstrated a favorable safety profile for ApidSOL™ technology in several species [68-70]. Furthermore, superior corneal delivery of CsA with CsA ApidSOL™ compared with Restasis® or oil-based CsA solutions has also been demonstrated [68]. A comparative ocular distribution study of CsA after repeated instillation (bid for 5 days) of 0.05% CsA ApidSOL™ or Restasis® into rat eyes demonstrated significant corneal drug deposition for the polymeric nanocarrier formulation CsA ApidSOL™, but not for Restasis®. More specifically, after the instillation of CsA ApidSOL™, 1540 ± 400 ng per gram tissue of CsA was recovered in the cornea, whereas the corneal CsA concentration after Restasis® instillation was below the limit of quantification (2 ng/mL) in all but one rat (n=6) [68]. This same trend was observed in another comparative study after repeated instillation (five times per day over 5 days) of 0.5% CsA ApidSOL™ and 0.5% CsA oily solution that reported CsA corneal concentrations of 6470 ± 1730 ng per gram tissue and 580 ± 110 ng per gram tissue for the polymeric nanocarrier formulation and oily solution, respectively [70]. While the lachrymal fluid concentration was similar after administration of 0.05% CsA ApidSOL™ or Restasis® (AUC 2339 ± 1032 min × µg/mL and 2321 ± 881 min × µg/mL), the polymeric nanocarrier formulation was better tolerated [68]. As determined by confocal laser scanning ophthalmoscopy, the tolerance profile of 0.05% CsA ApidSOL™ (four instillations (50 µL each) per day for 3 days) was similar to the profile of a saline solution (0.9%) [69].

These results are consistent with those observed with SFA formulations, demonstrating that micellar nanocarriers encapsulating CsA are able to enhance transcorneal penetration when compared with Restasis®-like emulsions, which predominantly affect the surface and require multiple instillations to penetrate into the cornea.

4.4. Others

A monocentric, randomized, noncomparative clinical trial including 92 patients (92 eyes) was conducted between May 2003 and June 2011 at the Shandong Eye Institute, China, to evaluate the effect of CsA on the prevention of rejection after high-risk corneal transplantation [71]. The CsA preparation used in the trial was a sustained-release implant made of poly(lactide-co-glycolide-co-caprolactone) (PLGC). CsA and PLGC were first dissolved in chloroform, and the solution was filter-sterilized, lyophilized and shaped into 2-mg cylinders (0.20 mm × 0.65 mm),

each containing 1.0 mg CsA. Implants were then injected into the anterior chamber during the keratoplasty surgical procedure, and outcomes were assessed at 6 months [71]. Treatment was successful in 81 eyes (88%), partially successful (no rejection after rescue medication) in seven eyes (7.6%) and failed (graft rejection) in four eyes (4.3%). The mean graft survival was 36.1 ± 17.7 months (range 12.3–61.6 months), and on average, the drug delivery system (DDS) degraded in 7.6 ± 4.3 months (range 5–13 months). There were no safety signals from the assessments of endothelial cell density and iris status. The CsA sustained-release DDS used in this study appears to be suitable for long-term prophylaxis of immune rejection after high-risk keratoplasty. The main advantage of this DDS is its ability to effectively deliver CsA over several months following a single minimally invasive injection.

5. Cyclosporine A formulations in academic research

Since the 2003 review of ocular CsA delivery systems by Lallemand *et al.* [4], there has been a steady increase in the number of academic publications on this topic, reflecting the strong medical need for new delivery options. In this section, we will first focus on topical delivery to treat front-of-the-eye conditions and then describe the ongoing research efforts to administer CsA via intra- or periocular injection procedures.

5.1. Topical formulations

The topical delivery of CsA can be divided into two main categories: 1) colloidal dosage forms based on submicron particles suspended in an aqueous phase and 2) other delivery systems, including suspended microspheres and solid dosage forms.

5.1.1. Colloidal vectors (nanometer size)

Colloidal vectors are submicron particles suspended in an aqueous solution and include several different types of nanoparticles. Nanoparticles have been tested as CsA carriers for more than 20 years; however, no clinical study has been conducted with nanoparticles to date, probably due to the complexity and costs of manufacture, as well as the low drug-loading capabilities of nanoparticles.

5.1.2. Chitosan nanoparticles

Chitosan is a cationic biopolymer that possesses mucus-like bioadhesive properties and a favorable ocular tolerance [72]. A proof-of-concept study in 2001 described chitosan as a potential colloidal carrier for CsA [73]. No further development was reported until Basaran *et al*. [74] made nanoparticles of chitosan by spray-drying a hydroethanolic solution of CsA and

chitosan, producing quite a low encapsulation rate (CsA concentrations in the final formulation of 0.05% and 0.125% w/w). The nanoparticles were resuspended in water and sterilized by autoclaving, with a procedural drug loss of about 7%. A pharmacokinetic study after single instillation of 500 µL in sheep eyes showed that the approximate CsA concentrations in aqueous and vitreous humors were 30–40 ng/mL, a level that is insufficient to induce immunosuppressive action [74]. However, the authors did not assess the CsA concentration in the cornea and conjunctiva to enable comparison with other delivery systems. The choice of sheep eyes for such a study is quite unusual, and the instillation volume (500 µL) was much larger than that typically used in humans (average drop size of several commercial topical ophthalmic solutions is approximately 40 µL, and overfilled liquid is eliminated) [75]. In summary, although the concept of chitosan nanoparticles as CsA delivery vehicles seems promising, additional data and comparative studies demonstrating their efficacy and safety when compared with other technologies are required to establish the utility of this drug delivery system.

Other cationic nanoparticles described by Hermans *et al.* [76] included a poly(lactic-co-glycolic acid) (PLGA) core coated with chitosan to increase precorneal residence time and drug absorption. This publication only demonstrated an *in vitro* toxicity study on human epithelial cells, and additional work is needed to evaluate its real potential.

5.1.3. Liquid crystalline nanoparticles

Chen *et al.* [77] described a liquid crystalline nanoparticles formulation containing CsA-loaded (1%) glyceryl monooleate and poloxamer 407. An *in vivo* (rabbit eyes) study of a prototype formulation demonstrated a significantly higher corneal penetration compared with oil solutions, and the Draize test did not show any signs of toxicity. Such nanoparticles have a high encapsulation rate due to their lipophilic core and use of compendial excipient. While these nanoparticles are simple to manufacture without the use of organic solvents, there is a significant cost associated with sterilization and validation of a full aseptic process. Further pharmacokinetic and pharmacodynamic data are needed to better understand the potential of this DDS.

5.1.4. Nanostructured lipid carriers

Shen *et al*. [78] have described a new drug delivery approach using lipid nanoparticles of 60–70 nm developed by solidification of a new excipient, thiolated polyethylene glycol monostearate, which is essentially a combination of a fatty acid and a surfactant. The oily core used in these

particles allows for a surprisingly high encapsulation of CsA (~1% w/w) in the formulation. When instilled into the eyes of rabbits (twice at 90-s intervals), high tear concentrations were maintained for up to 6 hours without any sign of irritation. This high retention time may be due to the bioadhesion of the nanoparticles to tear mucins via the thiol group present at the nanoparticle surface. Given their small size, these novel nanoparticles could be sterilized easily by filtration, a feature that makes them particularly interesting for ocular delivery. A limitation for this technology is the extensive and costly regulatory toxicity studies that will be required for this novel excipient, which could pose a significant hurdle to engaging an industrial partner. In a follow-up study, Shen *et al.* [79] undertook an extensive pharmacokinetic study in rabbit ocular tissues after instillation of 25 µL of the prototype. They demonstrated that these nanoparticles could deliver therapeutic tissue concentrations of CsA (cornea, conjunctiva, iris-ciliary body) for up to 24 hours post-instillation. Nonetheless, further investigation of these nanoparticles will be required to validate the utility of these nanoparticles as an ocular delivery vehicle.

5.1.5. Solid lipid nanoparticles

Gokce *et al*. [80] also described a slightly different approach from that of Shen *et al.* [78, 79] by developing solid lipid nanoparticles loaded with 0.05% CsA within a solid glyceryl dibehenate lipid core stabilized by poloxamer 188 and Tween 80. Interestingly, the integrity of the prototype suspension (polydispersity index and zeta potential) survived heat sterilization. In a non-comparative pharmacokinetic study in rabbit eyes, high CsA concentrations were detected in the aqueous humor, but no assessments of corneal and conjunctival CsA concentrations were done, making it difficult to interpret the comprehensive ability of these lipid nanoparticles as a DDS.

5.1.6. Poly-ε -caprolactone nanoparticles

Yenice *et al*. [81] pursued a poly-ε -caprolactone nanoparticle approach made by a nanoprecipitation technique using acetone as the organic solvent. This process differed from that used for previously developed particles [82] in that they were coated with hyaluronic acid (HA), a natural polymer, which improves interactions with ocular surface mucins. The HA coating was achieved by an electrostatic attraction between negatively charged HA polymer and the cationic surfactant (BAK) used in the formulation. The encapsulation efficiency of the poly-ε -caprolactone nanoparticles was similar to that of other nanoparticles, with a final CsA concentration of 0.1%. In a pharmacokinetic study in rabbits, corneal and conjunctival CsA concentrations were higher following four instillations (25 µL at 10-min intervals) of the nanoparticle suspension compared with instillation of a 0.1% CsA castor oil formulation [81].

Unfortunately, tolerability was not evaluated in this study, which is a concern since this formulation was prepared with an organic solvent and contains BAK. This is particularly important for patients with severe ocular conditions, given the potential for deleterious effects of BAK on the ocular surface.

5.1.7. Cyclodextrin nanoparticles

An interesting combination of α- and γ-cyclodextrins was described by Johannsdottir *et al*. [83] with the objective of developing a surfactant-free CsA solution. The α-cyclodextrin solubilized 0.05% CsA in an aqueous solution, whereas γ-cyclodextrin promoted the formation of nanoparticles. Nanoparticles based on these well-characterized sugars could be an alternative to other polymer nanoparticles. Further investigation of this approach is warranted to establish the utility of these nanoparticles. However, it can be noted that corneal irritation potential has been reported for α-cyclodextrins and RM-ß-cyclodextrins for concentrations above 4% and 5%, respectively. Other types of cyclodextrins, e.g. HP-ß-cyclodextrins and SBE-ß-cyclodextrins, appear to be safer, with no issues reported for concentrations up to 12,5% and 10% respectively.[84]

5.1.8. Mucoadhesive nanoparticles

A recent study by Liu et al [85] showed promising results for phenylboronic acid (PBA)-modified, CsA-loaded poly(D,L-lactic acid) + dextran (PLA-*b*-Dex) nanoparticles in the treatment of long-term experimental DED in mice. Once-weekly administration of these mucoadhesive nanoparticles eye drops (0.005 to 0.01% CsA) demonstrated prolonged ocular surface retention and effective treatment of dry eye conditions with up to 50- to 100-fold reduction in overall dosage of CsA compared with thrice-weekly Restasis®.

5.1.9. Liposomes

Liposomes were described many years ago for the topical administration of CsA [86] but failed to reach the market due to issues with large-scale production and stability. Of note, most production processes for liposomes use organic solvents, which must be removed in order to comply with the residual solvent specifications of the International Council for Harmonisation (ICH) on the Technical Requirements for Pharmaceuticals for Human Use [87]. In addition, organic solvents must be recycled, incurring additional costs. Since phospholipids naturally oxidize with environmental oxygen, liposomal products must be freeze-dried or packaged in an

inert atmosphere. Nevertheless, liposomes are academically interesting, and there is a lot of work in drug delivery with this type of preparation.

Recent work reports a novel formulation of liposomal CsA using a supercritical fluid of carbon dioxide (SCF-CO_2) method in order to address the challenges of liposome preparation [88]. In brief, excipients and CsA were solubilized in ethanol and submitted to SCF-CO_2 flow to eliminate solvent, and the resultant liposomes were then hydrated with water. These liposomes were smaller than conventional liposomes with similar drug loading. In a dry eye model in rabbits, the liposomes showed better tolerance, improved tear production in dry eyes and resulted in a higher CsA tear film concentration compared with Restasis®. This promising proof of concept deserves further development for testing on a larger scale [89].

Li *et al.* [90] investigated a CsA-loaded (0.1%) liposomal formulation coated with positively charged low-molecular-weight chitosan to confer bioadhesiveness to liposomes. A pharmacokinetic evaluation in rabbits (single ocular administration of 100 µL) showed that the chitosan-coated particles resulted in a significantly greater CsA penetration, especially into the cornea, compared with noncoated liposomes. As chitosan is usually considered to be nontoxic, this approach could be promising, although there remain uncertainties about the manufacturing and sterilization process and the stability of these liposomes. A safety study of liposomes suspended in a carbomer solution with a final CsA concentration of 0.2%, described by Mosallaei *et al.*, showed no significant toxicity; however, this study did not report any data on ocular penetration [91].

5.1.10. Micelles

Another way to solubilize CsA in water is to use micelles. Although this DDS was described a long time ago, it was not particularly successful due to the poor stability of such a dynamic system [4]. This approach is still being intensely investigated by Di Tommaso *et al.,* as explained in Section 4.3 above [66, 68-70], and there have also been several recent publications on this topic.

In 2014, Luschmann [92] described the use of three surfactant solutions of ceteareth-20 (5%), steareth-20 (5%) and macrogol (15)-hydroxystearate (10%) to solubilize CsA up to 0.1% w/v. These formulations are simple and easy to manufacture and can be sterilized by filtration. In a study on excised porcine cornea, these solutions showed a higher penetration rate than Restasis® or an oil solution of CsA [92], confirming the potential of micelles as a DDS, similar to descriptions by Kuwano *et al.*, who demonstrated an increased ocular bioavailability when CsA

was formulated as a micellar solution vs. an emulsion or an oily solution [40]. However, at surfactant concentrations of 5% and 10%, ocular irritation may be observed, especially after long-term use, and on-shelf stability data on these micelles have not yet been reported.

In a different study, Cholkar *et al*. [93] constructed micelles with hydrogenated castor oil-40 (Cremophor RH 40) and octoxynol-40 capable of solubilizing CsA in an aqueous solution up to 0.1%. The production method involved ethanol evaporation and film rehydration. Pharmacokinetic studies (single 35 µL dose and multiple doses of four instillations per day for 5 days) performed in rabbits showed therapeutic concentration of CsA s in all front-of-the-eye tissues (cornea, conjunctiva, sclera). These data once again demonstrate that a simple formulation can be useful in the ocular delivery of CsA. Unfortunately, there are no available data comparing this formulation to Restasis® or other vehicles or that show the potential irritation from this mixture of surfactants after several days of administration.

5.1.11. Other dosage forms

The following sections describe aqueous suspensions of microspheres and emulsions, as well as solid dosage forms such as lenses and inserts.

5.1.11.1. Microspheres

Wolska and Sznitowska [94] have recently investigated the microsphere approach to deliver CsA to the ocular surface. Microspheres contain a lipid matrix of triglycerides, glyceryl palmitostearate or glyceryl behenate stabilized by the surfactant Tween 80. A significant advantage of this type of formulation over other CsA DDSs is its high content of lipids, which allows for the encapsulation of up to 2% of CsA. The prototypes were shown to withstand heat sterilization at 121 °C for 15 min without degradation, and the typical particle size in this formulation was between 1 and 10 µm. Although this formulation has its advantages, the physical stability of a suspension containing particles of this size is still unclear. Moreover, the behavior of the particles after topical administration remains to be examined. It appears that a bioadhesion mechanism is not employed in this DDS, which may limit the residence time and ocular penetration of CsA when using this formulation. In addition, the lipids used for such formulations have a high melting point and are solid at room temperature, although this could be easily avoided by mixing the high-melting-point lipid with a low-melting-point lipid, for example MCTs or ethyl oleate, creating liquid lipid clusters within the particles wherein the drug could remain dissolved.

5.1.11.2. Emulsions/emulsions in gel

The first CsA emulsion was marketed as an ocular product in 2003, and three products are currently available: Restasis®, Lacrinmune® and Ikervis®. Emulsions and hydrogels have interesting features, and it is therefore expected that a combination of features of these two technologies would be advantageous for ocular delivery of CsA. Such a formulation may increase retention time, stability and potentially tolerability by wetting and lubricating the ocular surface. Gan *et al*. [95] have added hydrogels of gellan gum or Carbopol® 980 to an emulsion of 0.2% CsA (castor oil stabilized by macrogol (15)-hydroxystearate). The potential effect of these viscosifying agents is to enhance retention time on the ocular surface as well as increase ocular tissue penetration. In an *in vivo* pharmacokinetic study in rabbits, 100 µL was instilled twice a day for 1 week. At 24 and 32 hours after the final instillation, corneal CsA concentrations were significantly higher with the CsA emulsion-gel than with the control (a CsA emulsion similar to Restasis®, but without gel); however, conjunctival CsA levels were not significantly different. The emulsion-gel tested in this study contained a higher CsA concentration than the control (0.2% versus 0.05%) suggesting that the emulsion-gel had no significant advantage over a conventional emulsion in terms of ocular penetration.

Shen *et al.* [96] developed a 0.5% CsA emulsion gel using castor oil, ethanol and poloxamer 188. The gel also contained polycarbophil and water isotonized by glycerol, resulting in a white-colored, viscous and creamy product. When administered to rabbit eyes, the gel was nontoxic and tear CsA concentrations were significantly higher than concentrations achieved with a CsA castor oil solution. Although these preliminary results are promising, further studies are needed to establish the safety and efficacy of this formulation. In our opinion, the use of ethanol should be avoided due to potential safety issues, irrespective of whether it evaporates. The formulation should also be further characterized, since the size and zeta potential of the oil droplets could potentially influence the stability, retention time and CsA penetration of the emulsion. In addition, the sterilization of the formulation needs to be assessed. Finally, a pharmacokinetic study is warranted to validate the advantage of this dosage form over already commercially available products such as Restasis® and Ikervis®.

5.1.11.3. Hydrogels

Hydrogels are commonly used in ophthalmology to increase the retention time of a drug after topical administration. They act either by corneal bioadhesion or simply by their high viscosity, which limits their elimination from the eye surface. However, when viscosity is too high, administration of the gel to the ocular surface can be difficult and could cause discomfort for

patients. Therefore, *in situ* gelling systems have been developed that gel upon contact with the eye [97]. Wu *et al*. [98] have extensively studied a thermosensitive HA *in situ* gelling system for CsA delivery. A novel co-polymer was synthetized using HA-g-poly-isopropylacrylamide (2% w/w), capable of gelling at 32 °C, which is similar to the corneal surface temperature (34 °C). In addition, this co-polymer rearranges into microgels with lipophilic cores capable of encapsulating CsA. Drug loading was successfully achieved by combining the polymer solution with a methanol CsA solution. After evaporation of the solvent, any free CsA was removed by centrifugation and dialysis. While the Draize test revealed no significant irritation after a single instillation (25 µL) of the product, the long-term tolerability of this product is yet to be determined. In a pharmacokinetic study, six instillations of the prototype, an oil solution with the same concentration of CsA, and Restasis® were applied at 10-minute intervals to rabbit eyes. The tear, corneal and conjunctival CsA concentrations were similar between the hydrogel, castor oil and eye drop formulations at all time points except at 24 hours, wherein the hydrogel application resulted in significantly greater CsA concentrations in the cornea and conjunctiva. This DDS, while interesting, may not be a good candidate to progress into further stages of development given the poor pharmacokinetic results reported to date, as well as the complex process involved in manufacturing this new chemical entity.

5.1.11.4. Contact lenses

The use of contact lenses with active ingredients is a well-investigated DDS, with some experimental successes [99]. Kapoor *et al.* [100] used this approach to incorporate CsA into HEMA (2-hydroxyethyl methacrylate) contact lenses using two strategies: 1) by entrapping a CsA microemulsion via polymerization of poly-HEMA (p-HEMA) hydrogel and 2) by directly including CsA polyoxyethylene oleoyl ether micelles in the HEMA gel. *In vitro* studies showed that both prototypes released CsA for up to 20 days. Since p-HEMA hydrogels are capable of withstanding heat sterilization without damage [101], it can be hypothesized that the lens could also be potentially sterilized by moist heat without degradation. The results of this study were published in 2008, after which there have been no other publications to support this DDS [100].

Another team used silicone-based contact lenses to administer CsA [102]. In this approach, contact lenses were soaked in a 17 µg/mL CsA solution for 7 days, and drug loading through hydrophobic diffusion was driven by the difference between the partition coefficients of the solution and the hydrophobic lens core. At best, the lens could absorb 110 µg of CsA, and *in vitro* studies demonstrated sustained CsA release over 2 weeks, which could be prolonged up to 1 month with the addition of vitamin E to the lens. Despite these interesting results, as in the

previous example, no further updates in support of this DDS approach have been published to date.

Loaded contact lenses are an attractive approach because the manufacturing process is quite easy and the higher concentration of drug loaded into these lenses could support sustained release over several weeks. However, it is well known that the use of contact lenses in patients with dry eye or ocular surface inflammation can exacerbate symptoms or disease progression. Hence, CsA-loaded contact lenses may not be suitable for the treatment of DED or other associated diseases. Nevertheless, this DDS may be applicable to deliver CsA for the treatment of other ocular conditions such as corneal graft rejection and rosacea.

5.1.11.5. Ocular inserts

The first prototype for ocular inserts were described in the 1970s [103] and involved the insertion of a solid dosage form into the lower conjunctival cul-de-sac that released active drug for several hours to several days. However, despite their overwhelming potential, the initial commercialized products failed, and the use of this DDS was abandoned for many years due to the discomfort associated with placement of the insert experienced by patients. Recently, Gupta *et al*. [104] described a new ocular insert prototype for the ocular delivery of CsA. These inserts were made of nonbiodegradable p-HEMA and ethylene glycol dimethacrylate, which are both polymers commonly used in contact lenses and are capable of CsA loading up to 30%, with an average release rate of approximately 10–20 µg/day. Although promising, this research has not progressed beyond *in vitro* studies. An *in vivo* pharmacokinetic study evaluating the release rate and ocular bioavailability of CsA using this DDS is primarily needed for further assessment. Also, the development of an insertion device to easily administer and facilitate placement of the insert in the cul-de-sac by the patient would be beneficial. In our opinion, the insert should be made of a biodegradable polymer to reduce patient discomfort.

5.2. Injectable formulations

Despite significant advances in the topical delivery of CsA, the development of specific DDSs for sustained drug release or effective delivery of active drugs to the posterior segment still remains a challenge. Presently, delivery of CsA to the back of eye can only be achieved through invasive intravitreal, subconjunctival, intracameral, suprachoroidal or episcleral injections. Several different drug delivery approaches to specifically deliver active drug to the back of the eye have been tested, as discussed below.

5.2.1. Microspheres

He *et al*. [105, 106] described the *in vivo* evaluation of PLGA microspheres, approximately 50 µm in diameter, loaded with CsA formulated using a solvent evaporation process. When this formulation was injected into the vitreous humor in uveitis-induced rabbit eyes, the severity of the inflammation significantly decreased 4 weeks post-injection. There were also no signs of apparent toxicity [106]. These preliminary results are promising, though some critical technical issues in the formulation process, such as use of methylene chloride (a class 2 solvent regulated by the ICH), need to be addressed [87]. The processes used for sterilization of the particles and reconstitution into a homogeneous suspension must also be considered. Given the particle size, a 25-gauge needle, which is larger than the standard recommendation (30-gauge needle to limit the size of the perforation made in the sclera), may be required to inject these microspheres into the vitreous humor. Finally, it would be essential to determine the CsA release rate of this formulation in order to avoid the need for repeated injections. In our opinion, a 6-month period would be an ideal release rate to decrease the need for frequent injections.

5.2.2. Implants

Implants are solid dosage forms that act as reservoirs for sustained release of active ingredients. Presently, both biodegradable and nonbiodegradable implants are available that can be implanted in several sites within the eye. Acton *et al.* [107] tested a subconjunctival silicone implant of CsA in a wolf suffering from KCS. The successful outcome observed from this single case demonstrated the potential of this DDS and paved the way for further experimental work.

Episcleral injection (i.e. injection in the outer layer of the sclera) is not a common route of administration but is a promising approach that warrants further investigation. Kim *et al.* [108] developed a silicone-based episcleral CsA implant with a potential release time of 150 days [109]. After implantation into rabbit eyes, they noted that most of the front-of-the-eye organs (lacrimal gland, conjunctiva and cornea) and the aqueous humor retained therapeutic CsA concentrations for up to 6 months, but the CsA concentration in the vitreous humor remained below therapeutic levels. A pharmacodynamic study performed in dogs with KCS showed improvements in Schirmer's test scores compared with baseline and resolution of the signs of keratitis. The implant appeared to be safe and did not display any signs of toxicity [108]. A potential limitation of this implant is the nonbiodegradability of silicone, which could impact post-treatment status.

A recent retrospective study (2005–2013) of the episcleral CsA silicone implant in horses with immune-related keratitis reported that the implant was associated with long-term efficacy and was also well tolerated [110]. However, as described by Kim *et al.* [108], implantation is a surgical procedure, and the fate of the spent insert needs to be considered prior to implantation.

Biodegradable implants have the advantage of eliminating the need for implant removal and/or the potential risks associated with the accumulation of spent implants. Based on Ozurdex® technology (PLGA implant of dexamethasone, Allergan), an implant of 0.5 mg CsA was surgically placed in the anterior chamber of the eyes of rabbits [111]. The implant was efficient in maintaining a high corneal concentration of CsA for 3 months, providing proof of principle for use in preventing corneal graft rejection. However, no further work on this implant has been published since 2003.

An intravitreal CsA implant, 1.5 mm in diameter and 2 mm in length made of a new biodegradable copolymer, glycolide-co-lactide-co-caprolactone, developed by Dong *et al*. was surgically implanted in a rabbit model of chronic uveitis [112]. Post-implantation, a decrease in inflammation and an absence of any signs of toxicity were observed. Of note, 14 weeks post-implantation, 257.3 ng/mL of CsA was found in the vitreous humor, demonstrating that this new formulation could support sustained CsA release for up to 3 months. Since publication of the Dong *et al*. results in 2006, no additional work has been reported using this new co-polymer. One reason for this might be the regulatory hurdle of using a new polymer, which requires a complete toxicity evaluation prior to initiation of clinical trials on human subjects.

Gilger *et al*. [113] described the evaluation of a scleral implant of CsA (10% w/w) formulated using a pellet of CsA powder and a polyvinyl alcohol polymer. In this study, only deep scleral layer implantation in horses with equine recurrent uveitis effectively controlled uveitis when compared with implantation in the episcleral space. This implantation site has the advantage of bypassing the scleral barrier and directly delivering CsA to the choroid, where inflammation is concentrated. Despite the need for surgery, this device seems to have several advantages in term of sustained release, safety and achievement of therapeutic concentrations in the choroid.

A novel approach has been described by Eperon *et al*. [114] wherein a PLGA CsA- and triamcinolone-loaded implant was attached to an intraocular lens as a replacement lens for treating surgery-related uveitis. Cataract surgery was performed in a rabbit model of induced uveitis to evaluate the anti-inflammatory effect of the implant at different time points (up to 79 days). Signs of inflammation were significantly reduced at most time points. At the end of the

experiment, the implants retained 60% of the CsA that was originally loaded, suggesting that these devices could support sustained CsA release for several additional months. This approach is quite promising because it uses an approved polymer, the device can be sterilized by radiation, and it is implanted at the time of surgery, thereby obviating an additional invasive procedure. We hope to see further work using this approach in the near future.

6. **General conclusions**

Compiling all the formulation approaches covered by Lallemand *et al*. [4] and this review (Table 4), to date, more than 50 different approaches for the ocular delivery of CsA have been described, excluding several other technologies that are only described in patents. From this larger group, findings from 11 key studies are summarized in Table 5. CsA remains the most challenging compound to formulate in a suitable dosage form, stimulating extensive research efforts to devise optimal ocular DDSs. Even latanoprost, one of the most prescribed drugs in ophthalmology, did not stimulate as much research or the development of as many prototypes. We can say without a doubt that CsA is a textbook case in ophthalmic drug delivery.

It should be noted that it remains difficult to compare formulation types due to differences in protocols among prototypes, including the variability in CsA concentration (range: 0.05–2%), instillation volumes (range: 25–100 µL), modes of administration, instillation frequency and the controls used as comparators in each of these studies (typically Restasis® or an oil solution of CsA).Tolerance has not always been evaluated, although it remains a key factor in deciding the therapeutic chosen for ocular surface diseases. At a minimum, a Draize test with a single administration should be conducted, and ideally an ocular tolerance study of once-daily administration for 28 days should be performed. Furthermore, although sterilization is a prerequisite for a commercial eye drop, sterilization procedures are not systematically discussed in the development of emerging DDSs. For even the most promising academic prototypes, sterilization remains one of the main issues to be solved before securing approval for clinical testing of the product in human subjects. In our opinion, industrial partnerships should be initiated after completion of pharmacokinetic and toxicity studies to encourage product development into the regulatory approval and marketing authorization stages. Academic researchers should also be mindful of the need to patent their formulations to gain additional value for their work and facilitate appropriate industrial collaborations.

Restasis® in the US and Ikervis® in the EU are the two major and successful emulsion products that are currently marketed to treat DED and other ocular surface diseases. Promising new

technologies that could reach the market in the near future include polyaphron PADciclo™ from MC2BIOTEK and the semi-fluorinated alkane solution CyclASol® from Novaliq. The other drug delivery systems described in this review are still in the early stages of development.

Although not optimal, hospital preparations, especially 2% CsA oil solutions, will probably continue to be used routinely until an industrial product succeeds in providing equivalent amounts of CsA concentration to the eye. Looking into the future, it would be interesting to see which of these novel approaches will be the best in class and which will gain global marketing authorization as standard-of-care therapeutics for DED. Emulsions have thus far been the most reliable DDS, but this approach may be supplanted by one of the other formulations described in this review. Furthermore, for convenience, it is likely that daily administration formulations will be replaced by sustained-release formulations. Although the invasive administration required for most of the currently available sustained-release formulations is a drawback, future formulations should be developed such that self-administration allows for sustained drug release of at least several days to 1 week and ideally for 1–3 months. In the current list of possible formulations, bioadhesive ocular inserts can be self-administered and also have the potential to release CsA over a 1-week period. Surprisingly, this dosage form has not been investigated, probably due to previous commercial failures (e.g. Ocusert® [pilocarpine insert] and Lacrisert® [cellulose insert]).

One further question that remains to be evaluated is the most convenient site for injecting these formulations into the eye. For front-of-the-eye conditions, a subconjunctival injection is preferable, whereas for the back-of-the-eye diseases, an intravitreal injection remains the gold standard. Deep scleral injections may also be advantageous for back-of-the-eye delivery. With respect to the formulation, nanoparticles and microspheres are less invasive than larger implants and can release an active ingredient over a period of several months. In conclusion, we suggest that scientists focus on noninvasive sustained-release formulations that are stable, sterile and nontoxic and demonstrate high levels of safety, efficacy and tolerability.

Disclosures

F. Lallemand, M. Schmitt and J.S. Garrigue are employees of Santen SAS. J.-L. Bourges has nothing to disclose. R. Gurny is co-founder of APIDEL SA. S. Benita is a co-founder of Novagali SAS, now Santen SAS.

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Cyclosporine A delivery to the eye

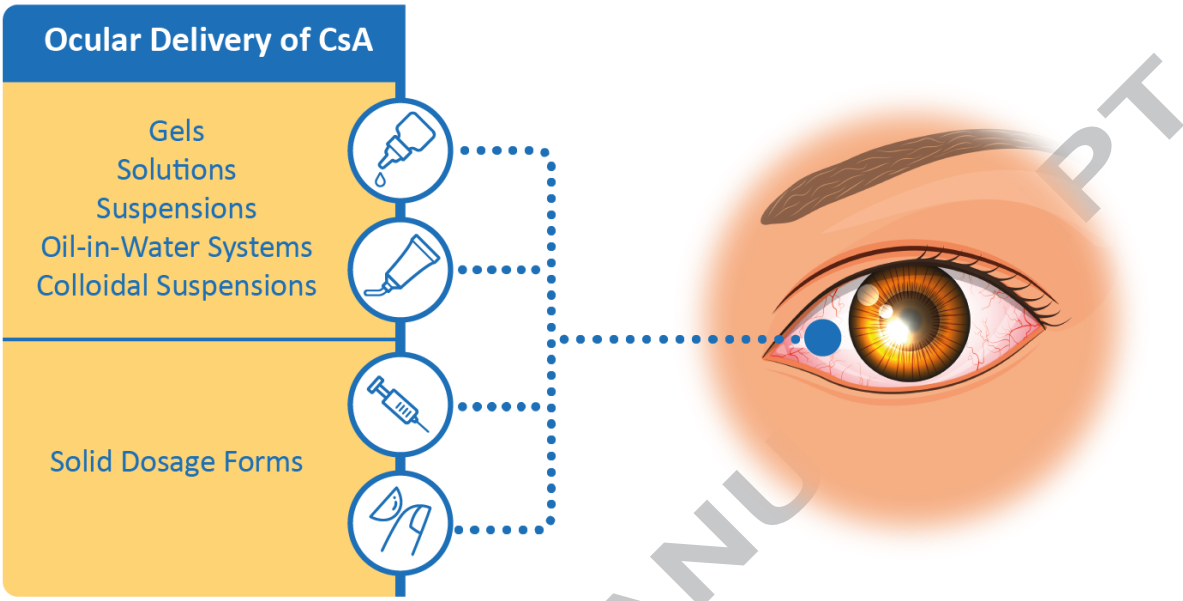

Table 1. List of ocular formulations of cyclosporine A marketed in different countries.

Product Name	Company	Dosage form	Dosing regimen	Indication	Region[a]	Marketed since
Restasis® (0.5 mg/mL)	Allergan	Anionic emulsion (UD)	bid	DED (KCS with presumed suppression of tear production)	US, Canada and 33 other countries	2003
Ikervis® (1.0 mg/mL)	Santen Pharmaceutical Co. Ltd.	Cationic emulsion (UD)	qd	DED (severe keratitis which has not improved with tear substitutes)	Europe	2015
Papilock mini® (1.0 mg/mL)	Santen Pharmaceutical Co. Ltd.	Solution (UD)	tid	VKC	Japan	2005
Modusik-A Ofteno® (1.0 mg/mL)	Laboratorios Sophia	Solution (MD)	bid	KCS with a functional decrease of lacrimal glands	Mexico, Chile, Colombia, Peru, Ecuador	2003[b]
Lacrinmune® (0.5 mg/mL)	Bausch & Lomb, Inc.	Emulsion (MD)	bid	KCS with a functional decrease of lacrimal glands	Argentina	NA
TJ Cyporin® (0.5 mg/mL)	Taejoon Pharm Co., Ltd.	Solution (MD)	bid	Ocular inflammation associated with KCS	South Korea	2003
Cyporin® (0.5 mg/mL)	Aristopharma, Ltd.	Solution (MD)	bid	Ocular inflammation associated with KCS	Bangladesh, Myanmar	NA
Cyclorin® (0.5 mg/mL)	Ibn Sina Pharmaceutical Industry Ltd.	Solution (MD)	bid	Ocular inflammation associated with KCS	Bangladesh	NA
Optimmune® (2.0 mg/mL)	Intervet, Inc. (Merck Animal Health)	Ointment (tube)	bid	Chronic KCS and superficial keratitis in dog	WW	1995

[a]Regions listed do not represent a comprehensive list of all countries with approved use of ocular formulations of cyclosporine A.

[b]Estimated date.

bid: twice daily; DED: dry eye disease; KCS: keratoconjunctivitis sicca; MD: multi-dose container; NA: not available; qd: once daily; tid: three times daily; UD: unit-dose container; VKC: vernal keratoconjunctivitis; WW: worldwide.

Table 2. Composition of licensed ocular formulations of cyclosporine A.

	Restasis®	**Ikervis®**	**Papilock mini®[a]**	**Modusik-A Ofteno®**	**Lacrinmune®[a]**	**TJ Cyclosporin®[a]**	**Optimmune®**
CsA (%)	0.05	0.1	0.1	0.1	0.05	0.05	0.2
Solubilizing agent/ enhancer	Castor oil	Medium-chain triglycerides	Polyoxyl-40 stearate Ethanol	Polyoxyl-40 stearate Polysorbate 80 Ethanol	Castor oil	–	Corn oil
Surfactant	Polysorbate 80	Tyloxapol Poloxamer 188 Cetalkonium chloride	–	–	Polysorbate 80	–	–
Preservative	–	–	–	Boric acid Sorbic acid	Potassium sorbate	–	–
Stabilizer	Carbomer copolymer type A		Sodium EDTA	Sodium EDTA			
Viscosity regulator	–	–	Hypromellose	–	Sodium hyaluronate	–	–
pH regulator	NaOH	NaOH	NaH_2PO_4 NaOH HCl	Sodium bisulfite	NaOH	–	–
Osmotic agent	Glycerol	Glycerol	NaCl	NaCl	Glycerol	–	–
Diluent	Water	Water	Water	Water	Water	Water	Petrolatum Lanolin Alcohol

[a]Approximate composition based on limited publicly available information.

CsA: cyclosporine A, EDTA: ethylenediaminetetraacetic acid, NaCl: sodium chloride; NaH_2PO_4: monosodium phosphate, NaOH: sodium hydroxide.

Table 3. Hospital-compounded preparations of cyclosporine A eye drops.

CsA starting material	Oil/solvents	Other components	Concentration CsA (% w/w)	References
Dilution of Sandimmune® (50 mg/mL injectable)	Artificial tears (Dulcilarmes®) Saline solution Olive oil Castor oil Corn oil MCT (Miglyol®) Peanut oil	Ethanol (± evaporated) ± polysorbate or Cremophor ± glycerol ± artificial tears	0.1% to 2%	Bontemps *et al.*, 2008 [47] Nourry *et al.*, 2006 [115] Minguez *et al.*, 1992 [116] Reinhard *et al.*, 1999 [117] Leconte-Astruc *et al.*, 2000 [118] Borel *et al.*, 2009 [119] Knagenhjelm *et al.*, 1999 [45]
Dilution of Sandimmune® (100 mg/mL oral)	Castor oil Olive oil Peanut oil		0.05% to 2%	Benitez del Castillo *et al.*, 1994 [46] Nourry *et al.*, 2006 [115] Minguez *et al.*, 1992 [116] Fauvel *et al.*, 2008 [50] Knagenhjelm *et al.*, 1999 [45]

CsA: cyclosporine A, MCT: medium-chain triglyceride.

Table 4. Summary overview of cyclosporine A delivery approaches.

Route of administration	Family	Type	Main excipient	Comment	References
Topical delivery	Solutions	Oily solutions	Various oils used as solvent for CsA (medium-chain triglycerides, olive oil, castor oil, peanut oil)	Relevant to majority of hospital-compounded formulations	[45, 46, 49]
		Semifluorinated alkane solution	Use of tamponading fluorinated alkane solution as solvent	Use of a safe and already-approved excipient	[61]
	Suspension	Microspheres	Lipid matrix of triglycerides, glyceryl palmitostearate or glyceryl behenate; stabilized by the surfactant Tween 80 in aqueous solution	Stability of suspension remains to be confirmed	[94]
	Oil-in-water systems	Emulsions	Composition described in Table 2	Commercially available products	[22, 37, 38]
		Emulsions in gel	Oil droplets dispersed in gellan gum or Carbopol 980®	No significant advantages compared to anionic emulsion	[95]
			Emulsion dispersed in carbophil	A pharmacokinetic study is needed	[96]

		Polyaphrons	Oil-in-water dispersion with a very large amount of oil	Enhancement of CsA ocular penetration Clinical stage product	[54], [59]
	Colloidal suspensions	Micelles	Composition described in Table 2	Commercially available products	[21, 23, 31]
			Micelles made of a new polymer, mPEG-hexPLGA, ApidSOL™	Promising *in vivo* results; Chemistry, Manufacturing, and Control data available (US FDA submission)	[65]
		Liquid crystalline nanoparticles	Nanoparticles of glyceryl monooleate and poloxamer 407	Simple and safe formulation that needs to be tested in pharmacokinetics	[77]
		Polymeric nanoparticles	Poly-ε -caprolactone nanoparticles coated with hyaluronic acid	Interesting bioadhesive potential, but tolerance needs discussion due to the presence of benzalkonium chloride	[81]
			Chitosan nanoparticles	Interesting approach, but advantage over other dosage form needs to be confirmed	[74]
		Nanoparticles of cyclodextrins	Nanoparticles of α- and γ-cyclodextrins including CsA disperse with polyvinyl alcohol	Only at formulation stage	[83]
		Nanostructured lipid carrier	New thiolated polyethylene glycol monostearate excipient	Promising approach, but regulatory status of the excipient needs to be	[78]

				confirmed	
		Solid lipid nanoparticles	Glyceryl dibehenate to form a solid lipid core stabilized by poloxamer 188 and Tween 80	Very interesting, but no further work since 2009	[80]
	Gel	Thermogelling gel	Hyaluronic acid-g-poly-isopropylacrylamide gelling at 32 °C	Status of this new excipient is unknown	[98]
	Solid topical dosage forms	Contact lenses	HEMA lens, 20-day release	Not suitable for injured corneal surface	[100]
			Silicone contact lenses, 2-week to 1-month release	Not suitable for injured corneal surface	[102]
		Ocular insert	Nonbiodegradable polymer HEMA and hydroxyl methacrylate loaded with up to 30% CsA	Promising approach; could be improved with a bioresorbable polymer	[104]
Injected products	Microspheres	Intravitreal microspheres	Microspheres of PLGA injected in the vitreous	Release (4 weeks) is too short compared with implants	[105, 106]
	Implants	Intravitreal implant	Biodegradable copolymer glycolide-co-lactide-co-caprolactone	New polymer; new pharmacokinetic profile, but regulatory status to be confirmed	[112]

		Scleral implant	Implant of CsA (10 % w/w) made of CsA powder and polyvinyl alcohol polymer	–	[113]
		Intraocular lens loaded	Implanted at the moment of lens replacement, no additional invasiveness than surgery, potentially several months of release	Innovative approach	[114]
		Subconjunctival implant	Silicone implant with sustained release	Nonbiodegradable	[107]
		Episcleral implant	Silicone implant with a potential release time of 150 days; very safe	Nonbiodegradable	[108, 110]
		Intravitreal PLGA implant	Same technology as used for Ozurdex® (PLGA intravitreal polymer matrix without a preservative)	Program apparently stopped in 2003	[111]

CsA: cyclosporine A, HEMA: 2-hydroxyethyl methacrylate, PLGA: poly(lactic-co-glycolic acid), mPEG-hexPLA: methoxy poly(ethylene)glycol-hexyl substituted poly(lactides).

Table 5. Summary of key studies.

Reference	Study type	Key Findings
Liang et al. [8]	In vitro (human corneal epithelial cells) + preclinical (rabbits)	CsA CE (Ikervis®) exhibited an ocular safety profile similar to that of Restasis® (0.05% CsA in castor oil) and hospital-compounded CsA formulations
Daull et al. [26]	Review (in vitro/preclinical/clinical)	Cationic oil-in-water eye drop nanoemulsions exhibit improved precorneal residence time due to electrostatic interactions between positively charged oil nanodroplets and the negatively charged ocular surface epithelium
Daull et al. [39]	Preclinical (rabbits)	CsA cationic emulsions were more effective than Restasis at delivering CsA to target tissues
Kauss Hornecker et al. [44]	Retrospective study of prescribing practices for hospital-compunded CsA eye drops (oil solution)	2% CsA solution was the most commonly prepared formulation, and this dosage was primarily used for the prevention of corneal graft rejection; lower-dosage strengths (0.5% and 0.05%) were typically used to treat DED, Gougerot-Sjögren syndrome, ocular rosacea, and severe allergies (atopic keratoconjunctivitis and VKC), as well as to prevent high-risk corneal graft rejection
Praestegaard et al. [58]	Preclinical (rabbits)	Pharmacokinetic analysis of PADciclo™ (a dispersion of polyaphrons encapsulating CsA within oily micrometer-sized aphrons dispersed in a hydrogel of carbomer) indicated negligible systemic CsA exposure; after multiple topical administrations, the conjunctival and corneal penetration of CsA was up to fivefold higher than that achieved with Restasis®
Dutescu et al. [61]	Preclinical (rabbits)	The penetration of 2 0.05% CsA SFA formulations (F4H5 and F6H8) into the anterior chambers of the eye after short-term application was significantly higher than that achieved with Restasis®
Di Tommaso et al. [68]	In vitro (rabbit corneal cells) + preclinical	CsA ApidSOL™, a novel aqueous formulation for the topical delivery of

	(rabbits, rats)	CsA based on polymeric micelles, demonstrated superior CsA delivery into rat corneas vs. Restasis® or oil-based CsA solutions
Shi et al. [71]	Clinical (randomized, noncomparative; 92 patients with corneal blindness who required corneal transplantation)	This study asessed the effect of CsA on the prevention of rejection after high-risk corneal transplantation using a novel, sustained-release drug delivery implant made of poly(lactide-co-glycolide-co-caprolactone) (PLGC). Based on data at 6 months, the implants appeared to be suitable for long-term prophylaxis of immune rejection, and there were no safety signals from the assessments of endothelial cell density and iris status.This system was able to effectively deliver CsA over several months following a single minimally invasive injection
Wolska et al. [94]	In vitro	This study investigated microspheres, which contain a lipid matrix of triglycerides, glyceryl palmitostearate, or glyceryl behenate stabilized by the surfactant Tween 80. A significant advantage of this type of CsA delivery system is its high lipid content, which allows for encapsulation of up to 2% CsA. Prototypes were shown to withstand heat sterilization at 121 °C for 15 min without degradation, and the typical particle size was 1-10 µm. The physical stability of a suspension containing particles of this size is unclear. Moreover, it appears that a bioadhesion mechanism is not employed, which may limit residence time and ocular penetration of CsA
Gupta et al. [104]	In vitro	This study described a new ocular insert prototype for delivery of CsA made of nonbiodegradable p-HEMA and ethylene glycol dimethacrylate; both are polymers commonly used in contact lenses and are capable of CsA loading up to 30%, with an average release rate of approximately 10–20 µg/day. Although promising, this research has not progressed beyond in vitro studies. An in vivo pharmacokinetic study evaluating the release rate and ocular bioavailability of CsA using this system is needed
Eperon et al. [114]	Preclinical (rabbits)	This study describes a novel approach

		wherein a PLGA CsA- and triamcinolone-loaded implant was attached to an intraocular lens as a treatment for cataract surgery-related uveitis. Inflammatory markers were significantly reduced at most time points. At the end of the experiment (79 days), the implants retained 60% of the CsA originally loaded, suggesting these devices could support sustained CsA release for several additional months. This approach is promising because it uses an approved polymer, the device can be sterilized by radiation, and it is implanted at the time of surgery

CsA CE, cyclosporine A cationic emulsion; DED, dry eye disease; HEMA, hydroxyethyl methacrylate; PLGA, poly(D,L-lactide-co-glycolide); SFA, semifluorinated alkane; VKC, vernal keratoconjunctivitis.