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Polymeric micelles for ocular drug delivery: From structural frameworks to recent preclinical studies

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Abstract

Effective intraocular drug delivery poses a major challenge due to the presence of various elimination mechanisms and physiological barriers that result in low ocular bioavailability after topical application. Over the past decades, polymeric micelles have emerged as one of the most promising drug delivery platforms for the management of ocular diseases affecting the anterior (dry eye syndrome) and posterior (age-related macular degeneration, diabetic retinopathy and glaucoma) segments of the eye. Promising preclinical efficacy results from both in-vitro and in-vivo animal studies have led to their steady progression through clinical trials. The mucoadhesive nature of these polymeric micelles results in enhanced contact with the ocular surface while their small size allows better tissue penetration. Most importantly, being highly water soluble, these polymeric micelles generate clear aqueous solutions which allows easy application in the form of eye drops without any vision interference. Enhanced stability, larger cargo capacity, non-toxicity, ease of surface modification and controlled drug release are additional advantages with polymeric micelles. Finally, simple and cost effective fabrication techniques render their industrial acceptance relatively high. This review summarizes structural frameworks, methods of preparation, physicochemical properties, patented inventions and recent advances of these micelles as effective carriers for ocular drug delivery highlighting their performance in preclinical studies.

Graphical Abstract

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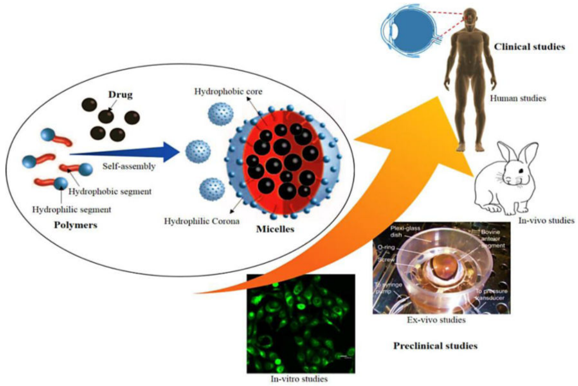
Conflict of Interest

The authors do not have any conflict of interest with regards to the subject matter.



Keywords

Ocular drug delivery; polymeric micelles; ocular barriers; dry eye syndrome; drug delivery; bioavailability

1. Introduction

The past decades have witnessed a significant progress in the development of nano-sized (1–200 nm) ocular drug delivery systems. Such increasing interest in nanomedicine may be attributed to the tremendous advances in nanotechnology, polymer chemistry and chemical engineering [1, 2]. However, additional research is required in the area of ocular drug delivery, particularly with regards to the delivery of hydrophobic compounds, nucleic acids and proteins, in order to improve their therapeutic outcomes and thus the quality of life of the patients [3, 4]. Hydrophobic NSAIDs such as indomethacin, ibuprofen, and diclofenac, indicated for inflammatory disorders, are an excellent example to demonstrate the need for improved ocular delivery. Although, in-vitro studies have suggested their pharmacological effectiveness, studies involving animal models and patients generally fail to achieve sufficient therapeutic activity [5, 6]. Such failure is most likely due to insufficient retention and accumulation at the target site resulting in suboptimal therapeutic levels. Additionally, significant amounts of intravitreally administered drugs accumulate inside healthy ocular tissues and can potentially lead to serious side effects, discomfort and blurred vision [7, 8].

Several nanomedicines have been formulated and evaluated for ocular drug delivery over the years. The most relevant formulations are depicted in Fig. 1. All of these have been designed keeping the following two key characteristics of nanomedicines in mind: (i) stable, efficient and reversible drug loading, as well as (ii) prolonged retention and circulation time. In the case of age-related macular degeneration (AMD), for instance, NSAIDs such as indomethacin and ibuprofen are known to be extensively utilized to reduce inflammation and cystoid macular edema [9]. However, because of their high hydrophobicity, both intravenous and intravitreal administrations are problematic and complicated. They are thus generally administered in combination with solublization enhancers, such as hydroxypropyl-β-cyclodextrin, diethylene glycol monoethyl ether (Transcutol P), n-octenylsuccinate starch, α-tocopheryl polyethylene glycol succinate, polysorbate 80 and tromethamine [10–13].

FDA approved polymeric implants for posterior segment drug delivery include Vitrasert (for CMV retinitis), Retisert (for uveitis), Iluvien (diabetic macular edema) and Ozurdex (for macular edema associated with uveitis and diabetes). Vitrasert and Retisert, based on the same delivery platform but with Retisert being slightly smaller in size, require sclerotomy at the pars plana region for implantation. On the other hand, Iluvien and Ozurdex are injected into the vitreous cavity via a 23–25 gauge needle. Since, Vitrasert, Retisert and Iluvien are non-biodegradable, the drug-depleted devices need to be surgically removed or may accumulate in the vitreous cavity as in the case of Iluvien. Taking frequent intravitreal implantation of these devices into consideration, many patients and insurance companies are taken aback by their price tags (USD $20,000 for Retisert and $2,000 for Ozurdex)[15]. Additionally, intravitreal administration of these implants requires skilled professional execution while carrying the risk of side effects potentially requiring patients to undergo cataract and/or glaucoma surgery as well as treatment with pressure lowering medications [11, 12]. Thus, exploring the feasibility of topical administration to deliver drugs to the posterior segment may drastically improve drug delivery in coming years, while minimizing costs and potential complications.

The physicochemical nature of nano-sized micelles also termed as "nanomicelles" consisting of a hydrophobic core and a hydrophilic shell, renders these spherical vesicles highly acceptable for passive drug delivery of hydrophobic compounds. Polymeric micelles (10–200 nm) are based on amphiphilic molecules or block copolymers which can generally self-assemble into organized core-shell/supramolecular structures in aqueous media at concentrations exceeding their critical micellar concentrations (CMC) [16]. On the other hand, low-molecular weight surfactant-based micelles exhibit higher CMC in contrast to polymeric micelles, leading to diminished stability and potential side effects. The potential of polymeric micelles to solubilize and stabilize hydrophobic compounds leads to their prolonged retention thereby improving therapeutic outcomes [17]. Nevertheless, micelle-based nanomedicines suffer from a myriad of problems when employed in in-vivo systems. Dilution in the blood stream as well as interactions with blood components (including plasma proteins such as albumin, fibrinogen, prothrombin and gamma globulins) lead to their premature disintegration in the systemic circulation. This substantiates the primary reason for the limited improvements in therapeutic efficacy of micellar nanomedicines in clinical trials when administered systemically [18, 19], while such issue would be minimal for topical ocular administration.

Core cross-linked polymeric micelles have evolved as a promising strategy to prevent the premature disintegration and release of therapeutic cargo. The cross-linking both in the core and corona of micelles can be based on covalent bonds, hydrogen bonding or π-π stacking. Unlike polymeric micelles where the drug molecules entrapped non-covalently are released in an uncontrolled manner, the covalent attachment in core cross-linked polymeric micelles allows stable drug retention and enables tailored release kinetics. The growing interest for the development of core cross-linked polymeric micelles for various disease conditions including ocular indications has led to an increasing number of cross-linked micellar based nanomedicines being designed and evaluated over last couple of years. Promising in-vivo results have entailed these cross-linked polymeric micelles to enter early stage clinical evaluations [14, 20].

In this review, we describe the basic principles for the preparation of commonly used polymeric micelles for ocular drug delivery. In addition, selected examples from the literature have been included to demonstrate significant improvements in the in-vivo performance of various drugs employing polymeric micellar based drug delivery systems. Besides, biodistribution, targetability, therapeutic efficacy and tolerability of these micellar formulations have been discussed. Finally, we report on the clinical translation and biomedical application of polymeric micelles. Together, the insights obtained indicate that polymeric micelles are highly effective delivery systems for intraocular drug delivery, (i) facilitating the formulation and entrapment of highly hydrophobic drugs, (ii) aiding drug administration, (iii) enabling controlled drug release kinetics, (iv) enhancing therapeutic efficacy, (v) reducing side-effects and and (vi) improving the in-vivo stability.

2. Challenges to ocular drug delivery

Efficient drug delivery to the ocular tissues faces a number of challenges due to the dynamic anatomy and the blood-ocular barriers (including blood-aqueous and blood-retinal barrier). From a drug delivery perspective, the eye can be divided into four parts: (i) the pre-corneal area (conjunctiva, eyelids); (ii) the cornea; (iii) the anterior segment (iris, ciliary body, lens) and (iv) the posterior segment (retina, vitreous cavity) [21].

The tear drainage rate constant from the pre-corneal area is 1.45 min^{-1} which results in 500 to 700 times greater drug loss from the ocular surface in comparison to the drug absorption rate into the anterior chamber [22]. This process eventually leads to less than 5% of the topically applied dose reaching the intraocular tissues. Additionally, the complex anatomy of the tear film with its outer oily layer retarding water evaporation, further impedes drug absorption into the cornea and sclera [23].

The cornea is an important route for drug absorption after topical application. It consists of five different layers, namely epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. The corneal epithelium plays a major role in limiting trans-corneal drug absorption with a drug permeability rate of only 10^{-7}-10^{-5} cm s^{-1} [24]. While small lipophilic drugs are passively transported via the transcellular pathway, hydrophilic drugs undergo restricted permeation through tight junctions via the paracellular pathway. The corneal stroma being hydrophilic, impedes transport of lipophilic drug molecules while hydrophilic molecules generally diffuse freely through the stroma. Overall, the efficacy of the trans-corneal route is limited by the relatively low absorption rate constants ($1–5×10^{-3}$ min^{-1}) [25, 26], with a molecular weight cut-off of 400–600 Da also playing an important role in the corneal permeability of drug molecules [27].

The high permeability of the human conjunctiva, on the other hand, can be attributed to its 17-fold larger surface area in comparison to the human corneal membrane. The larger paracellular spacing in conjunctival tissue (230 times greater than in the cornea cornea) facilitates passage of large hydrophilic molecules [28]. These characteristics have established the importance of the conjunctival-scleral pathway for intraocular delivery of macromolecules including proteins, peptides and nucleic acids. The trans-scleral route may also be utilized for the delivery of large molecules to the retina and vitreous, if suitable drug-delivery systems are employed. However, lymphatics and blood vessels present in the conjunctiva eliminate significant amounts of therapeutics via the systemic circulation [29].

Similar to the corneal stroma, hydrophilic drugs permeate through scleral matrix pores readily in comparison to lipophilic drugs. The molecular radius and charge of the drug molecule also greatly control the permeability across the sclera. Positively charged molecules appear to permeate the sclera poorly presumably due to their binding with the negatively charged scleral matrix [30]. Additionally, the blood ocular barriers contribute majorly, limiting drug entry into the posterior segment following systemic and periocular administration. The blood-aqueous barrier (BAB) is present in the anterior segment of the eye and restricts drug entry from the blood into the aqueous humor. However, the BAB is not considered a complete barrier because of the fenestrated capillaries present in the ciliary body stroma. These fenestrated vessels being the secondary source of plasma protein leakage to the iris also allow passage of small molecules to enter the iridial circulation [31]. The blood-retinal barrier (BRB) present in the posterior segment is further divided into inner and outer BRB. The inner BRB is composed of tight junctions between retinal capillary endothelial cells and is anatomically similar to the blood-brain barrier (BBB). The outer BRB is formed by the tight junctions between retinal pigment epithelial (RPE) cells. The greater density of tight junctions and pericytes in comparison to the BBB as well as the presence of glial cells, render the inner BRB highly effective in limiting transport of drugs from the blood into the retina [32, 33].

3. Routes of ocular drug administration

Topical drug administration in the form of eye drops is the most common and convenient method for the treatment of anterior segment diseases such as dry eye, conjunctivitis, and keratitis and may also be effective to some extent for the treatment of posterior segment diseases. However, pre-corneal drug loss remains a major drawback for topical administration. Owing to the complexity of the ocular anatomy and blood-ocular barriers, intraocular injections have advanced as common methods for ocular drug delivery. Intracameral administration involves direct injection of the drug solution into the anterior chamber. It is generally utilized after cataract surgery as well as for the treatment of anterior segment diseases such as fungal and bacterial keratitis. Nevertheless, this method fails to deliver therapeutic concentrations to the posterior segment [34]. This is primarily due to the penetration of the drug against the aqueous humor flow in the eye. Thus, intravitreal injections have generally been employed to achieve therapeutic drug concentrations in the posterior segment of the eye. However, a high-level risk of ocular complications and retinal toxicity remains as major drawback of this delivery route [35]. In comparison to intravitreal injection, the risk of ocular pain, infection, endophthalmitis or hemorrhage is comparatively low with periocular (e.g. subconjunctival, sub-tenon, retrobulbar, and peribulbar) injections. Additionally, periocular injections may provide longer duration of action in comparison to intravitreal injections due to the possibility of injecting lager volumes (up to 1 mL in comparison to 100 μL intravitreally) and the drug reaching to the posterior segment via three different routes: the transscleral pathway, the systemic circulation through the choroid and the anterior pathway through the tear film, cornea, aqueous humor, and the vitreous humor. However, relatively frequent injections may still be required for periocular administrations

[36]. Hence, it is extremely important to develop a non-invasive drug delivery system that can maintain therapeutic drug concentrations at the site of action over prolonged periods in a safe and effective manner without blurring of vision or causing any discomfort. Fig. 2 depicts various routes for ocular drug administration.

4. Ocular delivery pathways of micelles

After topical application of an eye drop, a drug is anticipated to follow either the corneal or the conjunctival-scleral pathway to reach posterior segment tissues [37]. The hydrophilic stroma which constitutes 85–90% of the cornea, acts as a rate limiting barrier for topically applied hydrophobic drugs [38]. Such limitation can be overcome by encapsulating hydrophobic drugs into the lipophilic core of highly water soluble polymeric micelles. Polymeric micelles, being extremely small, can penetrate through the cornea and/or the alternative conjunctival-scleral pathway after topical application. The higher conjunctival-scleral surface area allows lateral diffusion of such polymeric micelles to reach the posterior segment of the eye [39]. Nano-sized micelles with their hydrophilic corona assist in scleral transport of the micellar-drug construct through the aqueous pores/channels. In addition, the scleral pathway also minimizes the chances of drug washout into the systemic circulation by the conjunctival blood circulation and lymphatics. From the posterior segment, the polymeric micelles may be further engulfed by RPE cells by endocytosis to generate therapeutic concentrations in posterior ocular tissues [40–42]. Such tissue absorption and cellular uptake depend on the surface charge and size of the micelles [43]. Fig. 3. shows a schematic illustration of the penetration of a polymeric micellar formulation applied in the form of a topi cal eye drop to the posterior ocular tissues.

5. Polymeric micelles

Polymers, one of the most versatile classes of materials, have changed our day-to-day lives over the past decades. The ability to achieve either spatial or temporal control of drug delivery has led to their heightened potential in the field of polymer and pharmaceutical sciences. To date, various polymer based nanocarriers, including nanoparticles (NPs), liposomes, solid-lipid nanoparticles (SLNs), and dendrimers, have been exploited for the management of posterior ocular diseases [44, 45]. However, the majority of these formulations are administered via intracameral, intravitreal and periocular injections to overcome the ocular barriers with frequent injections generally required which may result in adverse effects[35]. Recently, polymeric micelles have shown growing evidences as a potential nanocarrier to overcome such limitations and provide therapeutic drug concentrations in the ocular tissues of the anterior and posterior segments following topical administration [46, 47].

Most of the polymeric micelles used in drug delivery consist of amphiphilic di-block (hydrophilic-hydrophobic) polymers, tri-block (hydrophilic-hydrophobic-hydrophilic) polymers, graft (hydrophilic-hydrophobic) and ionic (hydrophilic-ionic) copolymers. For the majority of these systems, poly(ethylene glycol) (PEG) is the primary hydrophilic segment [48]. Fig. 4. illustrates applications of polymeric micelles fabricated from PEG-*b*-

poly(amino acid) copolymers in delivering a variety of therapeutic entities including hydrophobic drugs, pDNA, siRNA, proteins, peptides and photosensitizers.

5.1. Principles of micelle formation

The polymeric units self-assemble into a nanoscale aggregate (10–200 nm), known as micelles having a hydrophobic core and a hydrophilic corona. Such self-assembly is favored by a thermodynamic process. The hydrophilic chains cover the hydrophobic core to avoid their direct contact with water and thus reduce the interfacial free energy of the polymer-water system. Micellar formation confides upon the reduction of the interfacial free energy [50, 51]. The degree of self-aggregation generally depends on the polymer chain concentration, the properties of the drug or any targeting agents, and the mass and composition of the copolymer backbone [52]. Depending upon the molecular weight of the block copolymers, micelles can have different shapes including spherical, cylindrical and star-shaped structures [53, 54].

5.2. Critical micelle concentration (CMC): a key factor in micellization

The minimum polymer concentration required to form micelles is termed as CMC. Below the CMC, insufficient numbers of polymeric chains are uniformly distributed in the solution as monomers. With an increase in the concentration, more polymeric chains are absorbed at the interface. The concentration, at which the interface becomes saturated with polymeric chains is known as the CMC. Above this point, an increase in the polymer concentration in solution results in the formation of micelles [55, 56]. Polymeric micelles (10^{-6} –10^{-7} M) usually exhibit CMC values 1000 times lower than that of low-molecular-weight surfactant based micelles (10^{-3}–10^{-4} M), with a low CMC value indicating increased stability of the micellar structure in an aqueous solution [57, 58]. For topically administered micellar ophthalmic formulations, the CMC is a critical factor. It regulates the chances of premature drug release from the formulation due to constant tear dilution and nasolacrimal drainage. Dispersity (D), also known as polydispersity index (PDI), is another important factor that can influence the shape, stability and overall performance of the micellar formulation. The kinetics of copolymers which exist between the micellar and non-micellar state are also influenced by the dispersity of the polymers. Therefore, block copolymers with monodisperse core-shell structures, having a low dispersity ($D<1.2$), are preferred for the development of controlled release systems [59, 60]

5.3. Effect of polymers on micelle properties

Polymeric micelles are primarily fabricated using amphiphilic diblock copolymers. However, in some cases, triblock copolymers and graft copolymers have also been utilized for micellar preparation to achieve prolonged drug release, enhanced blood circulation time and targetability [61–64]. The hydrophilic outer part of polymeric micelles can be composed of PEG, poly(ethylene oxide) (PEO), poly (acryloylmorpholine), poly(trimethylene carbonate), or poly(vinyl pyrrolidone) [65, 66]. A mixture of polymers, such as PEO and polyelectrolytes can also be incorporated into the hydrophilic part [67]. Hydrophilic polymers, such as PEG, impart stealth properties to polymeric micelles thereby avoiding their uptake by the reticular endothelial system (RES).

Block copolymers such as PEO-poly(L-amino acids) provide additional advantage to modify the properties of core forming blocks. For example, the carboxyl functionality of the poly(L-aspartate) block is amenable for chemical conjugation with a drug [68]. The hydrophobic core of polymeric micelles is usually made up of Pluronics (poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)), poly(L-amino acids) (such as poly(L-aspartate) and poly(L-glutamate)), polyesters (such as poly(glycolic acid), poly(D-lactic acid) and poly(D,L-lactic acid)), copolymers of lactide/glycolide, and poly(ε-caprolactone) [56]. Table 1. lists the various polymers used in the preparation of micelles for ocular drug delivery.

5.4. Polymeric micelle structures

The preparation of polymeric micelles can be divided into three categories namely (i) polymer–drug conjugates, (ii) drug-encapsulated carriers and (iii) polyion complex micelles.

5.4.1. Polymer–drug conjugates—Micelle forming polymer-drug conjugates are developed through hydrolysable chemical bonds between the functional group(s) of the polymeric backbone and the drug (Fig. 5.). To date, poly(ethylene oxide)-b-poly(ester) and poly(ethylene oxide)-b-poly(amino acid) block copolymer based conjugates have been extensively studied for effective drug delivery applications. Thes polymers may have several functional groups providing various sites for the conjugation of a number of drug molecules to one polymeric chain allowing efficient delivery of therpeutic doses. [87–91].

5.4.2. Drug-encapsulated carriers—The solublization and stable encapsulation of drug into polymeric micelles can primarily be achieved by chemical and physical methods. Chemical methods involve covalent core cross-linking of the drug with the polymers consisting of side-chain or end-group-functionalized block copolymers. An important factor to be considered using chemical methods is that the reactive groups of the polymers should be sufficiently hydrophobic or low in number so that they do not interfere with the formation of monodisperse micelles. The most commonly employed methods for preparing core cross-linked polymeric micelles are: (i) radical polymerization, used for micelles containing polymerizable groups in their core; (ii) addition of bifunctional crosslinkers, used for micelles containing reactive groups in their core; and (iii) disulphide bridges, used for micelles containing thiol groups. The last method allows for stimuli-responsive disintegration and drug release. Although chemical crosslinking allows significant improvements in circulation kinetics, biodistribution and target site accumulation of micelles, the series of chemical reactions involved may sometimes be challenging and complicated. On the other hand, physical methods including (i) direct dissolution, (ii) dialysis, (iii) oil-in-water emulsion, (iv) solvent evaporation, (v) co-solvent evaporation and (vi) freeze-drying methods (Fig. 6.) are much simpler and practical.

5.4.2.1. Direct dissolution method: The direct dissolution method is the simplest technique for the preparation of drug-loaded polymeric micelles. Copolymers along with drugs are mixed in water at or above the CMC to self-assemble into drug-loaded micelles. However, this technique is associated with low drug-loading which can be enhanced by increasing the temperature of the system before the addition of the copolymer. Preparation of a thin film of drug before the addition of the polymer into the system can also result in higher drug loading [92].

5.4.2.2. Dialysis method: The drug along with the block copolymer are dissolved in a water-miscible organic solvent (such as N,N-dimethylformamide) and the resultant solution is dialysed against water [93–97] (Fig. 6A). During the dialysis process, the organic solvent is replaced by water which induces self-association of block copolymers and the entrapment of drug. The semipermeable membrane of the dialysis bag keeps the drug encapsulated polymeric micelles inside. At the same time unloaded or free drug remains outside the dialysis bag. However, this method is only suitable for lab scale production, while incomplete removal of the free drug from the polymeric assemblies is another drawback. Alternatively, tangential flow filtration (TFF) is a fast and simple method that can be used for scalable manufacturing processes of polymeric micelles [98].

5.4.2.3. Oil-in-water emulsion method: The drug is dissolved in a water-immiscible organic solvent (such as chloroform or methylene chloride), followed by addition of this organic to the aqueous phase under vigorous stirring. The polymer may be dissolved in either the organic or the aqueous phase and the organic solvent is then removed by evaporation (Fig. 6B) [99–101].

5.4.2.4. Solvent evaporation method: Drug and polymer are dissolved in a volatile organic solvent. A thin film of drug/polymer is formed at the bottom of the flask after the evaporation of the organic solvent. This film is then reconstituted in an aqueous phase by vigorous shaking (Fig. 6C). This method can be applied for micelle-forming block copolymers having high hydrophilic lipophilic balance (HLB) values so that the polymer film can be easily reconstituted in an aqueous medium [102–104].

5.4.2.5. Co-solvent evaporation method: Drug and polymer are dissolved in a volatile water-miscible organic solvent (co-solvent) followed by addition of the aqueous phase (non-solvent for the core-forming block) which triggers the formation of micelles and drug entrapment. The organic co-solvent is evaporated at the end resulting in drug loaded polymeric micelles (Fig. 6D) [105–108].

5.4.2.6. Freeze-drying method: This method is applicable for freeze-dryable organic solvents such as tert-butanol which can dissolve the polymer and the drug. The drug/polymer solution is then mixed with water, freeze-dried and reconstituted with isotonic aqueous media. This method is suitable for large scale production. However, it is applicable only to block copolymers and drugs that can be solubilized in tert-butanol (Fig. 6E) [109].

5.4.3. Polyion complex micelles—Electrostatic interactions between oppositely charged polymer/drug combinations result in polyion complex micelles. This method is suitable for different therapeutic moieties that carry charge including peptides and DNA. Upon neutralization of the charge, the core-forming segment of the block copolymer can induce self-assembly of the polyion complex and further stabilization of the micelle [110–113].

5.5. Drug release from polymeric micelles

Drug release from polymeric micelles mainly depends upon (i) the design and method used for their preparation; (ii) the structure of the micelle-forming block copolymer and the drug; (iii) their physicochemical properties; and (iv) the localization of the drug in the polymeric micelles. Fig. 7. shows various modes of drug release from polymeric micelles. Drug release from polymer-drug conjugates generally follows two mechanisms, (i) dissociation of micelles followed by drug cleavage from the polymeric unimers or (ii) drug cleavage inside the micellar structure followed by diffusion out of the carrier (Fig. 7A). Drug release from drug-loaded micellar carriers is usually preceded by diffusion (Fig. 7B), whereas drug release from polyion complex micelles is triggered via ion exchange in physiological media (Fig. 7C).

Apart from these basic drug release mechanisms, instant, sustained, pulsed or delayed drug release can be achieved by modifying the chemical structure of the micelle-forming block copolymers which will ultimately modify the physicochemical properties of the core/shell forming blocks. For example, sustained or delayed drug release from the carrier can be achieved by enhancing the hydrophobicity and rigidity of the micellar core which limits the movement of water and free ions to the micellar core in micelle-forming drug conjugates and polyion complex micelles [68, 114].

The lower rate of micellar dissociation, the diffusion of drug and the overall drug release from micellar carriers can also be achieved via crosslinking of the micellar core structure and the induction of strong hydrophobic interactions or hydrogen bonds between the core-forming blocks and the drug [115–117]. The incorporation of hydrophilic or stimuli responsive groups to the core structure may be another avenue to provide an instant or pulsed mode of drug delivery [116, 118–121].

6. Pre-clinical development, in-vitro and biodistribution studies of polymeric micelles

6.1. Polymeric micelles prepared using the direct dissolution method

Hafner and coworkers developed ~29nm-sized Pluronic® F127 (denoted as F127) and chitosan (CH) cationic polyelectrolyte based micelles for dexamethasone (DEX) using the direct dissolution method with the F127/CH micellar systems releasing approximately 67% of the loaded drug within the initial 3 h into a release medium containing water and SDS (0.03% w/w). Systems with higher CH content demonstrated more rapid drug release in comparison to lower CH content indicating the significant effect of CH on micelle stability. Such an effect may be driven by a combination of different factors including (i) the hydrophobic interactions between the hydrophobic sequences of F127 (poly(propylene oxide), PPO) and CH, (ii) solubilization of the CH hydrophobic sequence and (iii) enhanced solubility of the PPO sequence by CH. Additionally, the higher initial release rate could be attributed to the passive diffusion of DEX from the F127/CH micelle core/shell interface while the second phase presented the release of DEX incorporated into the inner core of the F127/CH micelles. Significantly higher apical-to-basolateral permeability (apparent permeability coefficient; P_{app}) values were obtained for the F127/CH micelle systems in comparison to CH-free F127 micelles in Caco-2 cell monolayers. TEER values across caco-2 cell monolayers were monitored to investigate the effect of F127/CH micelle systems on tight junction opening.

The more pronounced decrease in TEER (up to 55–72%) caused by all F127/CH micelle systems in comparison to F127 alone (up to 82%) may be attributed to the interaction of positively charged CH with the negatively charged glycoproteins on the cell surface or the interior of the tight junctions. In-vivo biodistribution studies in rabbits revealed an increase in bioavailability of DEX (AUC 162.8±11.23) in comparisons to commercial DEX (0.1% w/v eye drop (AUC 67.5±9.42) and F127 alone (AUC 115.9±8.31) which can be attributed to the synergistic enhancement of transcorneal permeation caused by F127 and CH. Additionally, the intraocular pressure (IOP) was measured to determine the DEX ocular bioavailability with high steroid levels generally resulting in increased IOP. Micelle systems required longer time for the IOP to return to its normal circadian value (t_{min}) indicating prolonged circulation and thus pharmacological effects of DEX [83]. A similar study by the same group using a F127 micellar solution of pilocarpine in rabbits demonstrated an enhanced miotic response compared to an aqueous solution of the drug again proving its better bioavailability [122]. In addition, Chetoni *et al.* reported improved ocular bioavailability and a faster onset of indomethacin action in male New Zealand albino rabbits using a Poloxamer 407 micellar solution in comparison to the marketed indomethacin product [123].

Shamma and co-workers compared the synergistic behavior of mixed polymeric micelles composed of linear and branched poly(ethylene oxide)-poly(propylene oxide) with single micellar systems for more effective encapsulation of lornoxicam (LX), a hydrophobic NSAID. The binary systems included different weight ratios of highly hydrophilic poloxamers, Synperonic1 PE/P84 and Synperonic1 PE/F127, as well as the hydrophobic poloxamine counterpart (Tetronic1 T701). Mixtures of poloxamers and poloxamines have previously shown to improve the physical stability of efavirenz-loaded micelles [124]. Herein, all mixed nanomicellar systems (mean diameter ~169 nm) demonstrated an at least seven-fold increase in LX solubility compared to an aqueous solution (0.0318 mg/mL). In addition, high encapsulation efficiency was observed for these mixed micelles. In-vitro release studies demonstrated zero-order kinetics for mixed micelles with the lowest T701 concentration, while diffusion-based release kinetics were observed with equal or higher T701 concentrations. Approximately 60% of the drug was released within 6 h with no signs of redness, inflammation or increased tear production in the rabbit's eye. Histopathological studies revealed intact corneal epithelium and endothelium with similar stromal thickness to that of normal corneal tissue following micellar formulation instillation. This study further confirmed that the polymeric nonionic surfactants are relatively harmless to the eye in comparison to their cationic, anionic, or amphoteric counterparts and hence have found widespread applications in ophthalmics [80].

Civale *et al.* reported on a series of polyhydroxyethylaspartamide (PHEA) based copolymers, bearing PEG and/or hexadecylamine (C16) in the side chains as a potential carrier for ocular drug delivery. The PHEA backbone hereby served as an inert polymer, while incorporation of long alkyl chains (C16 or a combination of C16 chains and hydrophilic chains such as PEG) imparted lipid membrane interaction and drug complexation abilities. In-vitro permeability studies across primary cultured bovine conjunctival (BCEC) and corneal epithelia (BCoEC) cells conferred higher permeation for PHEA-C16 and PHEA-PEG-C16 drug-loaded micelles in comparison to simple drug solutions or suspensions. In particular, PHEA-PEG-C16 demonstrated to be the best permeability enhancer achieving almost 1.5- and 6-fold higher permeability in comparison to dexamethasone in BCEC and BCoEC cells respectively. In addition, drug loaded into PHEA-C16 and PHEA-PEG-C16 micelles was partially protected from degradation (50%) compared to the control formulations. In-vivo bioavailability studies in rabbits further confirmed that PHEA-PEG-C16 micelles not only acted as solubilizing enhancer but also provided higher drug bioavailability in comparison to the aqueous drug suspension. The AUC obtained after ocular administration of PHEA-PEG-C16 micelles (9494 ng/(ml*min)) was 40% higher than that of the control (5976 ng/(ml*min)) [73].

Alpha-lipoic acid (ALA) is an antioxidant indicated for the treatment of diabetic keratopathy and retinopathy. ALA-loaded Soluplus® (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol copolymer) based polymeric micelles were reported to enhance ALA solubility by ten-fold and may exhibit favorable effects on corneal residence time of the drug in comparison to currently available eye drop solutions. This indicates the potential of such polymeric micelles for efficient treatment of diabetes-associated corneal diseases such as keratitis, dry eye and ocular herpes [125].

6.2. Polymeric micelles prepared using the solvent evaporation method

CH-based nanosystems are often used due to their strong interactions with the negatively charged corneal surface leading to reversible disruption of corneal epithelial tight junctions and thus increasing corneal penetration. The polycationic nature of CH renders such nanosystems highly favorable for adhering to the ocular mucosa and thus facilitating the entry of drugs into superficial layers of the cornea and conjunctiva. Shi and coworkers developed a block polymer composed of cationic chitosan (CS) and methoxypoly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL), which could self-assemble into cationic micelles (100–150 nm) to solubilize hydrophobic drugs such as diclofenac (DIC). Previous studies with nearly neutral DIC/MPEG-PCL nanoparticles exhibited rapid clearance from corneal tissues. Herein, similar in-vitro release profiles for DIC from commercial eye drops and the DIC/MPEG-PCL-CS nanosuspension were obtained. A two-stage release, with an initial rapid release may be beneficial in achieving therapeutic drug levels in a short time, followed by a slower sustained release of the drug to maintain the minimal effective concentration. In-vitro corneal penetration studies demonstrated enhanced penetration of DIC from a DIC/MPEG-PCL-CS nanosuspension relative to the DIC commercial eye drops. A linear relationship between the cumulative penetration of DIC from both formulations over time indicated passive diffusion as the primary mechanism of DIC transport into the cornea. Corneal permeation was further confirmed in-vivo using Nile Red (NR) as fluorescent agent. The NR/MPEG-PCL-CS nanosuspension resulted in higher fluorescence intensity in the corneal endothelium than the NR aqueous solution 60 min after instillation. In addition, the DIC/MPEG-PCL-CS nanosuspension demonstrated higher concentrations of DIC (C_{max}) in the aqueous humor of male New Zealand albino rabbits in comparison to commercial DIC eye drops (2.3-fold higher AUC than commercial DIC eye drops) indicating greater drug retention and improved drug bioavailability (Fig. 8.) [126].

PEG is often added to increase the density of the hydrophilic corona in polymeric micelles and thus efficiently reduces irritation and cytotoxicity when in contact with human corneal epithelial cells. A representative example for PEGylated micelles is demonstarted by the use of monomethyl poly(ethylene glycol)-poly (ε-caprolactone) for entrapping pimecrolimus by Fan *et al.* The polymeric micelles displayed a size of ~37 nm and demonstarted a significant increase in tear production on day 20 in mice in comaprison to artificial tears, an oil-based eye drop, and a thermosensitive hydrogel containing the same drug. The reason may be ascribed to continous release of pimecrolimus from the polymeric micelles leading to blockage of cytokine transcription and thus promoting tear secretion. Histopathological examination of the cornea revealed minimal alteration in corneal epithelial thickness and number of inflammatory cells (Fig. 9.). Most importantly, polymeric micelles enhanced the water solubility of pimecrolimus up to 2485-fold while maintaining its activity [127]. All these findings exemplify the potential of polymeric micelles as an ophthalmic drug delivery system. Another example of polymeric micelles has recenlty been reported by our laboratory. In this study, di-block polymers based on methoxy poly(ethylene glycol) and poly(ε-caprolactone) (mPEG-PCL) were used to form approximatetly 27 nm-sized polymeric micelles using the solvent evaporation method. Transscleral permeability studies in excised rabbit sclera indicated a 2.5-fold increase in permeability of DEX with nanomicelles in comaprison to a DEX suspension. This study inidicated the potential of polymeric micelles in improving the bioavailability of DEX in the uvea following topical administration [128].

Guo and colleagues made important contributions towards improving the corneal and conjunctival permeation of therapeutic drugs by utilizing a new polymer with amphiphilic properties to overcome issues related to high manufacturing costs, toxicity and instability. In their work, about 73 nm sized micelles based on polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PVCL-PVA-PEG, Soluplus®) combined with CsA were prepared. Of the incorporated drug, almost 93 ± 7% remained encapsulated in the nanomicelles after 3 months of storage at 25 °C under light protection. In-vitro uptake after 5 min of incubation with human corneal epithelial cells (HCECs) revealed an 11.26-fold increase in coumarin-6 (Cou-6) uptake with nanomicelles in comparison to free drug in solution. Confocal laser scanning microscopy results demonstrated much lower fluorescence intensity in the cell cytoplasm with free Cou-6 in solution in comparison to the nanomicelles. Intracellular trafficking of nanomicelles revealed their rapid transport to apical early endosomes (AEE) (in less than 10 min) and then to late endosomes (LE) (Fig. 10A). A significant amount of Cou-6 was detected in lysosomes and the endoplasmic reticulum (ER) after 60 min indicating lysosome- and ER-trafficking pathways for Cou-6-loaded nanomicelles. Corneal permeation studies in New Zealand white rabbits demonstrated higher CsA levels in the nanomicelle group (6.32–168.26%) in comparison to an oil-based commercial ophthalmic formulation with no ocular irritation (Fig. 10B). However, CsA concentrations could not be determined in the aqueous humor in any of the groups as the concentrations were below the LOD (9 ng/mL) [129].

Our laboratory has also developed 10–40 nm sized clear mixed micellar formulations of DEX using D-α-tocopherol polyethylene glycol-1000 succinate (Vit E TPGS) and octoxynol-40 (Oc-40). Nanomicellar formulations improved DEX solubility by about 6.3-fold with minimal toxicity in rabbit primary corneal epithelial cells (rPCEC). In-vitro studies demonstrated drug release for up to 4.2 days preventing the need for frequent dosing [131]. In another study, a similar formulation was developed for rapamycin. In-vivo studies revealed high rapamycin concentrations in the retina/choroid (362.35 ± 56.17 ng/g tissue) indicating the efficacy of mixed nanomicellar formulations for back-of-the eye delivery after topical administration [132]. We have also developed around 22 nm sized micellar formulations of CsA using a blend of polymers including polyoxyethylene hydrogenated castor oil 40 (HCO-40) and Oc-40. Ocular tissue CsA distribution studies in New Zealand white albino rabbits with single and multiple dosing revealed CsA levels in the following order: cornea > iris-ciliary body > aqueous humor > lens. CsA levels were also detected in conjunctiva > sclera > retina/choroid > vitreous humor. The high CsA levels in the retina/choroid (53.7 ng/g tissue) indicated that nanomicelles were able to deliver CsA not only to the anterior but also to the posterior ocular tissues including the retina. Additionally, tissue distribution studies revealed that the nanomicelles are possibly transported through conjunctival-scleral pathway [133]. This formulation has demonstrated 10 mm improvement in tear production and significant reduction in signs of ocular surface inflammation in 455 dry-eye disease patients at 12 weeks in a Phase 2b/3 clinical trial and is currently undergoing Phase 3 confirmatory studies (NCT02688556)[134].

6.3. Polymeric micelles prepared using the co-solvent evaporation method

Mondon and colleagues synthesized polymeric micelles of amphiphilic copolymers based on methoxy-poly(ethylene glycol) (MPEG) and hexyl-substituted poly(lactides) (hexPLA). It was observed that the CMC of MPEG–hexPLA micelles decreased with an increase in hexyl groups which in turn facilitated better stability upon dilution. Up to 6 mg/mL of CsA was efficiently incorporated into MPEG–hexPLA micelles, an almost 500-fold increase in CsA water solubility [135]. At concentrations of 10 mg/mL, MPEG–hexPLA incorporated twice the amount of CsA compared to MPEG–polycaprolactone (MPEG5000 g/mol–PCL13,000 g/mol) micelles (approximately 1.3 mg/mL) as reported by Aliabadi *et al.* [136].

In a similar approach, 37.4±0.1 nm sized CsA loaded polymeric micelles were prepared by methoxy poly(ethylene glycol)-poly(hexyl-lactide) (MPEG-hexPLA) copolymers by the co-solvent evaporation method. In this particular example, immunohistochemistry studies showed that caspase 3, IL-DNase II and AIF were not activated, indicating that negligible caspase-dependent or independent apoptosis was induced after treatment with these micelles (Fig. 11.). Additionally, a clonogenic test was utilized to assess the toxicity of the MPEG-hexPLA formulation. No toxicity was observed and cells continued to divide following the mitotic cycle. In-vivo evaluation of ocular tolerance in rabbit eyes using confocal laser scanning ophthalmoscopy revealed minimal corneal surface damage indicating the tolerance of MPEG-hexPLA micelle formulations [77].

The hydrophilic nature of mucin layer covering the corneal and conjunctival epithelium in the eye render it as a protective barrier against the diffusion of macromolecules, microbes and hydrophobic molecules [137]. Mucoadhesive polymers have recently gained importance and are extensively explored to increase the bioavailability of drug in the immobilized mucin layer. Phenylboronic acid (PBA) has exhibited ability to form high-affinity complexes with 1,2-cis-diols unlike other natural mucoadhesive polymers such as chitosan, polysaccharides and thiomers to achieve desirable release kinetics [138]. Sheardown and coworkers developed a series of mucoadhesive block copolymer micelles based on PBA using reversible addition−fragmentation chain transfer polymerization (RAFT) (Fig. 12A). Such polymers are designed for targeted delivery of CsA to the ocular mucosa. CsA loaded-pLA-b-p(MAA-PBA) (LMP) copolymeric micelles, where pLA stands for poly(D,L-lactide) and MAA for methacrylic acid, exhibited a size range of 36±1.0 – 50±5.7 nm. All CsA-loaded micellar formulations demonstrated a release profile with initial rapid release (35–40%) within 24 h followed by a non-linear release of 74–80% for ~14 days. Compared to chitosan, all PBA based LMP formulations showed excellent in-vitro mucoadhesion suggesting their potential to improve ocular bioavailability. PBA micelles didn't exhibit significant cell cytotoxicity. However, clustered HCEC cell growth was obtained probably due to the adhesive nature of the micelles, hence mediating their interaction with cell surface mucins. 10 days of daily instillation of micellar solution drop into the right eye of rats did not show any visible effect on corneal health (Fig. 12B). Corneas remained transparent, healthy, and free from visible hyperemia or inflammation further demonstrating the excellent ocular biocompatibility of PBA based LMP micelles [139].

Similarly, Li *et al.* demonstrated the efficiency of 28 nm-sized triblock copolymer poly(ethylene glycol)-poly(ε-caprolactone)-g-polyethyleneimine (PEG-PCL-g-PEI) based polymeric micelles both in-vitro and in-vivo. These positively charged micelles allowed enhanced corneal permeation by prolonging the ocular residence time and sustained the interactions with the negatively charged cell membranes. Two-photon laser scanning microscopy was utilized to visualize the penetration behavior of drug molecules across the cornea of BALB/c mice. A time-dependent corneal stroma localization was obtained indicating the potential of polymeric micelles to overcome the epithelial barrier and release the incorporated drug into the stroma. Furthermore, PEGylated shells of these polymeric micelles exhibited higher stability and improved the solubility in both hydrophilic and hydrophobic drugs leading to their effective penetration into the amphipathic cornea [43]. In another study, Meyer *et al.* investigated indocyanine green/2-hydroxyethyl 12-hydroxyoctadecanoate (ICG/Kolliphor HS15) micellar formulations in an animal model of laser-induced choroidal neovascularization. High fluorescence intensities were observed within laser induced lesions for ICG micellar formulations in comparison to the conventional aqueous ICG solution. Immunohistochemical analysis of retinal and choroidal flat mounts revealed accumulation of Dye700 (dicarbocyanine analogue to ICG)/HS 15 within the laser lesions. The signal intensity at the choroid was much stronger compared to the retina. In addition, the micellar formulation prevented clustering of ICG aggregates. This led to reduced quenching of the dye and thus generated a more intense fluorescence signal. Therefore, the prepared micellar formulation may be effective in the management of choroidal and potentially also retinal diseases [140]. Chen and co-workers developed a MPEG-PCL based micellar formulation of diclofenac. A 17-fold increase in in-vitro transcorneal penetration and a two-fold increase in the AUC_{0-24} h in-vivo in comparison to diclofenac phosphate buffered saline (PBS) solution indicated the potential of diclofenac loaded MPEG-PCL micelles in improving ocular drug bioavailability [141].

7. Gene delivery using polymeric micelles

Although there is a lot of interesting and innovative work being published on the preparation of polymeric micells, there is only a small number of reports on improved in-vivo gene delivery. Liaw and coworkers developed a non-ionic triblock copolymer (PEO-PPO-PEO) based on propylene oxide (20%) and ethylene oxide (80%) for gene delivery into ocular tissues. These polymers have been widely used as a solubilizing, wetting, and emulsifying agent with relatively low toxicity in medical and pharmaceutical set-ups. So far, the poor efficacy of nonviral gene carriers in-vivo has been attributed to the low sensitivity of the carrier to serum, the instability of the DNA-carrier complex, and the poor cellular uptake of the complex.

The hydrophilic PEO chains in PEO-PPO-PEO provide a palisade region which inhibits protein/serum absorption in-vitro, and improves the stability in the blood stream. In addition, PEO type carriers enhance passive transport and avoid liver degradation when administred orally or systemically. A size range of 160 nm with a zeta potential of −4 mV indicated the potential of DNA/PEO-PPO-PEO polymeric micellar complexes to enhance junctional permeation and prevent enzyme digestion/interaction with serum in tears. Moreover, the size of PEO-PPO-PEO/DNA polymeric micellar complexes diminished significanlty (394 ± 38 nm to 155 ± 44 nm) due to the condensation of plasmid DNA in the presence of the polymers. Intense lacZ gene expressions in ocular tissues including the cornea and the RPE of mice and rabbits were reported on days 2 and 3 after topical administration of cytomegalovirus (CMV) lacZ plasmid/PEO-PPO-PEO polymeric micelles (Fig. 13.). Quantitative analysis of lacZ gene expression revealed an 28 and 38% increase for nude mice and rabbits respectively. In addition, utilization of EDTA and cytochalasin B appeared to enhance the transfection properties of plasmid/PEO-PPO-PEO polymeric micelles by opening the tight junctions paracellular pathway and thus accelerating permeation. This examplified non-ionic PEO-PPO-PEO polymeric micelles as a promising tool for topical gene delivery with low toxicity and high payload stability [142].

8. Hydrogel containing cross-linked micelles

Previous sections have clearly demonstrated that polymeric micelles can substantially improve drug stability and in-vivo ocular biodistribution leading to enhanced accumulation at the target site. Although, polymeric micelles in the form of eye drops are able to exhibit higher ocular biodistribution, around 75% of the total volume is lost from the precorneal pocket within the first 2–6 min due to blinking, nasolacrimal drainage and systemic absorption by the conjunctiva [143]. In-situ gelling system based micellar formulations have recently gained significant interest for effective ocular delivery and are being extensively exploited for further research and development (Table 2.). Clinical studies have shown the potential of soft contact lens based drug delivery systems in achieving significantly higher drug penetration across corneal layers compared to eye drops and subconjunctival injections. For example, it was recently demonstrated by Schultz *et al.* that significant drug levels can be achieved in posterior ocular segment tissues after sustained drug release from drug-loaded contact lenses [144]. Various methods are available for loading drugs into contact lenses. Conventionally, hydrophilic drugs are loaded into poly(2-hydroxyethyl methacrylate) (pHEMA) contact lenses by soaking the lenses in a buffer drug solution. Loading of hydrophobic drugs is achieved either by soaking the lenses in a drug-ethanol solution or by direct entrapment of drug during the polymerization of HEMA. However, the delivery of the payload is typically only increased over a short period of several hours.

In this respect, Lu *et al.* developed nanosized core cross-linked methoxy(polyethylene glycol)-block poly(ε-caprolactone) (MePEG-b-PCL) based micelles incorporated into a pHEMA hydrogel. An extended release profile of a model compound was obtained through the micelle-pHEMA system. However, some of the model compound present at the core-corona interface of the micelles was prematurely released during the photopolymerization of the hydrogel monomer [20]. Following up on this, the same group coated the MePEG-b-PCL micelle core with a silica shell prior to its incorporation into the pHEMA hydrogel in order to mitigate the drug release during the hydrogel gelation process. Such pHEMA-based hydrogels loaded with silica shell cross-linked micelles (SSCMs) were tested for sustained delivery of dexamethasone acetate (DMSA) into the eye. These 20 nm sized micelles with rod-like morphological characteristics were prepared by the solvent evaporation method (Fig. 14A). The in-vitro drug release profile from DMSA-loaded SSCMs demonstrated a biphasic distribution with a burst release over the first 8 h followed by a release of 6% per day over 6 days (Fig. 14B). In contrast, hydrogel containing DMSA-loaded SSCMs provided sustained release of the drug for periods up to 30 days (Fig. 14C). This indicates that the cross-linking with silica coating plays a significant role in limiting premature drug release from the SSCM-hydrogel system. In addition, the higher transparency and wettability of the SSCM-hydrogel system renders it a suitable platform for sustained delivery of therapeutic agents from soft contact lenses [74].

An interesting study conducted by Li and co-workers demonstrated a micellar supramolecular hydrogel formulation composed of mPEG block polymer and α-cyclodextrin (α-CD) through host-guest interactions for topical ocular drug delivery. Such a supramolecular hydrogel may be easily broken by shear forces and thus can exhibit a gel-sol transition upon external stimulation. The unique ability of CDs to form host-guest inclusion complexes with their inner cavities render them highly suitable for a variety of polymeric chains to form supramolecular hydrogels. Herein, DIC-loaded micelles were formulated which were then cross-linked with α-CD in a controlled manner to form a micellar supramolecular hydrogel. Gelation experiments demonstrated rapid gelation with higher concentrations of α-CD and mPEG-PCL which might be attributed to the higher cross-linking. In addition, incorporation of DIC into mPEG micelles did not alter the gelation time.

However, an increase in the PCL segment length enhanced the gelation time from a few seconds to about 1 h. In-vivo corneal surface studies revealed no corneal epithelial damage at 24 h after hydrogel instillation in comparison to untreated eyes (control) indicating superior ocular tolerance of hydrogels (Fig. 15A). In-vitro release studies demonstrated 95% DIC release from DIC/mPEG$_{2000}$-PCL$_{3000}$ micelles within 12 h (Fig. 15B). In contrast, micellar supramolecular hydrogels exhibited sustained drug release over 216 h. Pharmacokinetic studies in rabbit aqueous humor indicated a 2.37-fold higher C_{max} and a 1.98-fold higher AUC for the DIC-loaded micellar supramolecular hydrogel in comparison to DIC/mPEG-PCL micelles (Fig. 15C). The higher ocular bioavailability may be attributed to the prolonged corneal retention by the cross-linked hydrogel and enhanced corneal permeation by the micelles [146].

Polymeric micelles in ocular gels have also been applied for the delivery of antifungal agents for the management of fungal keratitis. Jaiswal *et al.*, for instance, reported the preparation of pH sensitive Itraconazole-loaded Pluronic based micelles in a Carbopol gel for controlled delivery to the anterior segment of the eye. The approximately 79 nm sized micelles demonstrated in-vitro permeation of 90.28 ± 0.31% within 8 h. Ex-vivo permeation studies across goat cornea exhibited 41.45 ± 0.87% permeation with zero-order kinetics (r^2= 0.994). In-vitro antifungal activity studies revealed a higher zone of inhibition (18.65 ± 1.46 mm) produced by the micellar formulation in gel in comparison to that of Itral® eye drops (14.42 ± 1.96 mm). Approximately 40 times higher mucoadhesive strength was observed for the formulation in comparison to the ocular shear stress which might explain its potential in resisting the shear stress during reflex blinking. Such a significant increase in the mucoadhesive properties of the formulation at a pH above 5.5 could be attributed to hydrogen bonding leading to sol-to-gel transition. Furthermore, Hen's Egg Test on Chorioallantoic Membrane (HET-CAM) revealed better tolerance of the formulation when compared to 0.3 mL of positive (1 M NaOH) and negative control (0.9% NaCl). Corneal histological sections indicated no damage to the structural integrity of the corneal epithelium further explaining the potential of such controlled release pH-sensitive ocular formulations for the treatment of fungal keratitis [70].

Wang and group recently developed a thermosensitive nanogel of muscone (a poorly water-soluble drug) by the reverse micelle/positive micelle (RM-PM) method using poloxamer 407 (Fig. 16.). It has been demonstrated previously that gel formulations including 20–30% poloxamer exhibit no ocular irritation and toxicity [78]. The muscone nanogel demonstrated a narrow particle size distribution ranging from 55–90 nm with high drug loading (97–100%). In-vitro transcorneal penetration studies revealed a 3.35-fold higher apparent partition coefficient (P_{app}) of the muscone nanogel in comparison to muscone eye drops.

Sodium fluorescein solution was quickly eliminated from the surface of the cornea 5 min after instillation into the lower conjunctival sac of the rabbit's eye indicating its short residence time. However, fluorescein incorporated into nanogels resulted in the greatest fluorescence intensity 10–30 min after instillation confirming the increased residence time and the sustained release by the nanogel. Pharmacokinetic studies using ocular microdialysis revealed that the C_{max} and $AUC_{0\text{-}6}$ of muscone in the rabbit's aqueous humor after instillation of the muscone nanogel were 6.5 and 6.33 times higher than muscone eye drop respectively (Fig. 17.). This confirmed the potential role of nanogels in prolonging precorneal residence time and thus improved ocular drug bioavailability [75].

9. Pre-clinical efficacy studies of polymeric micelles

Convincing evidence for improved ocular biodistribution of polymeric micelles has been provided by Tommaso *et al.* The group developed an aqueous formulation of MPEG–hexPLA based polymeric micelles (approximately 32 nm) for topical delivery of CsA for dry eye treatment. Results obtained from in-vitro uptake studies in rabbit primary corneal cells revealed micelles to be internalized by the corneal cells and delivered the cargo inside the cells after 24 h (Fig. 18A). This suggested the efficacy and biocompatibility of the colloidal formulation. No alteration in tear production and evaporation by the micellar formulation was shown by Schirmer and osmolarity tests respectively (Fig. 18B). Ocular distribution studies with the colloidal system in rats revealed therapeutic CsA concentrations (1540 ± 400 ng CsA/g tissue) in corneal tissues, while corneal CsA concentrations with a commercially available CsA emulsion (Restasis) were below 2 ng/mL. Precorneal retention kinetics in rabbits revealed a CsA concentration of 327 ± 262 ng/mL for the micelle formulation and 142 ± 70 ng/mL for Restasis in the lachrymal fluid 3 h post instiallation. This indicated that the CsA micelle formulation can efficiently deliver CsA into the cornea thus reaching therapeutic conecntrations [147].

In a subsequent study, Tommaso and co-workers evaluated the MPEG–hexPLA based polymeric CsA micelles with reagrds to ocular penetration, distribution and efficacy. Corneal transplantation studies in rats revealed significantly lower mean scores for corneal transparency, edema, and neovascularization in comparison to the control group at day 13 with lower scores signifying a better clinical outcome. At the end of the study, a 73% mean success rate was reported for the rats treated with the CsA micelle formulation in comparison to 25% for the control group (Fig. 19A). CsA concentrations after topical treatment with the micellar formulation in healthy rats were reported to be 6470 ± 1730 and 890 ± 610 ng CsA/g tissue in cornea and iris-ciliary body respectively. Obtained CsA concentrations were significantly higher in comaprison to 580 ± 110 ng CsA/g tissue in the cornea and below the LOQ (2 ng/mL) in the iris-ciliary body for the oil formulation (Fig. 19B). Higher CsA concentrations were also reported in the spleen (40 ± 20 ng CsA/g tissue) and in the cervical ganglia (330 ± 290 ng CsA/g tissue) of healthy rats explaining the potential role of CsA micelle formulation in inhibiting the activation of T-lymphocytes involved in the graft rejection cascade [39]

10. Potential polymeric micellar formulations for clinical translation

Various polymeric micellar formulations have been patented to date exhibiting great potential for proof-of-concept efficacy and safety as novel ophthalmic micellar formulations. However, for successful clinical translation, a few critical steps are necessary. Obtaining preclinical proof of efficacy and pharmacokinetic data in various animal models is a prerequisite before going into clinical trials. Besides the therapeutic response, systemic exposure and biodistribution in animal models should be reported. Direct head-to-head comparisons of the therapeutic efficacy of various related formulations are strongly recommended. In addition, in-vitro and in-vivo safety and tolerability profiles should be generated to determining a safe dose range for clinical evaluation. A thorough characterization including impurity profiling and methods for upscaling production is imperative for successful clinical translation. Table 3. lists recent patent inventions related to polymeric micellar formulations for effective ocular drug delivery with most of these still being under preclinical development.

11. Future prospects and conclusion

Over the past decades, polymeric micelles have shown potential for enhancing the solubility and therapeutic efficacy of hydrophobic drugs. The lipophilic core of polymeric micelles allows better encapsulation of hydrophobic drugs due to hydrophobic interactions while the hydrophilic corona renders them highly water soluble. In addition, they protect drugs from degradation and thus provide enhanced stability. Due to their small size and hydrophilic nature, micelles can efficiently permeate the corneal barriers and produce therapeutic concentrations both in the anterior as well as the posterior segment of the eye. They can improve the residence time of hydrophobic therapeutic molecules in ocular tissues and thus improve their pharmacokinetic and pharmacodynamic profiles. Another major advantage of polymeric micelles over other nanocarriers is that they involve easy scale-up processes and low cost of production.

Apart from the various routes employed for micellar ocular delivery including intravitreal, intracameral, periocular and systemic injections, topical application is considered to be the safest and most convenient method. However, the majority of the topically administered drug is lost due to blinking, nasolacrimal drainage and systemic absorption by the conjunctiva. To overcome such pre-corneal loss, polymeric micelles can be cross-linked with in-situ gelling systems. Such cross-linking will not only render the system more stable but also prolong the residence time of the micellar formulation on the ocular tissues thus providing sustained therapeutic effects over a prolonged period of time. Interestingly, such micelle-loaded in-situ gelling systems have also shown potential in protecting therapeutic molecules especially small hydrophobic molecules from degradation resulting in their enhanced intravitreal half-life. Based on the preclinical success, a number of polymeric micelles have recently entered clinical trials. To further enhance their clinical success, micelles can even be engineered for the delivery of commonly used macromolecules. Moreover, polymeric micelles responsive to external stimuli such as pH, temperature, enzymes, light and ultrasound may exert their smart function by delivering the loaded drugs in a site-directed and controlled manner.

In this review, many examples of the potential benefit of polymeric micelles for efficient delivery and thus treatment of various ocular diseases have been described both in-vitro and in-vivo. Polymeric micellar formulations that have the potential of undergoing clinical translation have also been discussed. These examples clearly illustrate the potential of these polymeric constructs as novel delivery platforms for efficient treatment of ocular diseases in the future.

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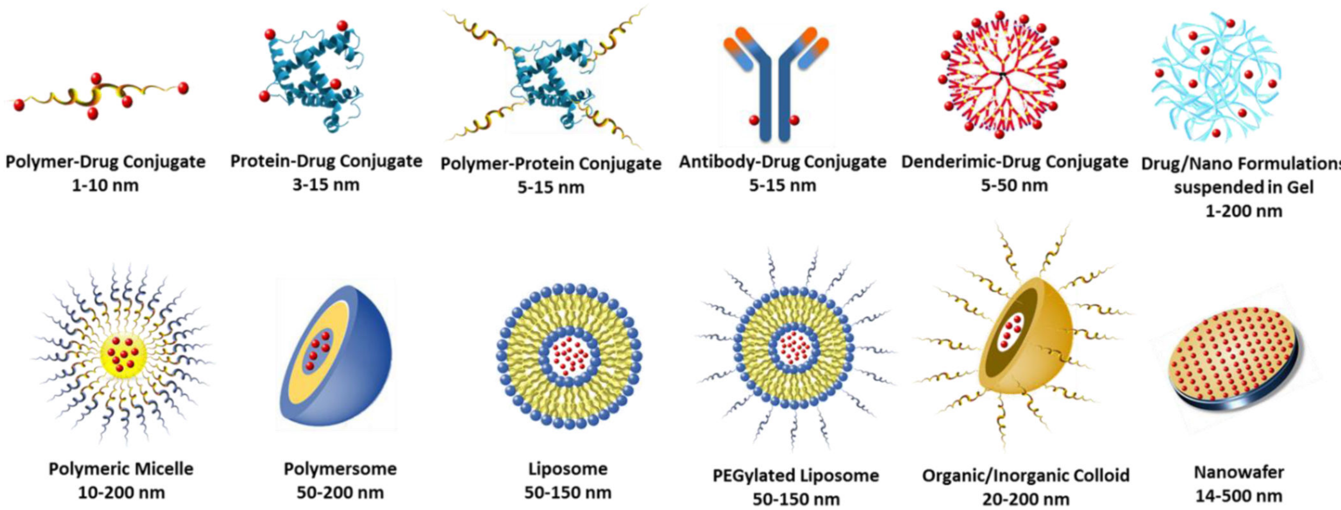

Fig. 1.

Schematic depiction of the most relevant nanomedicine formulations for ocular delivery [14]. Reprinted with permission from Elsevier.

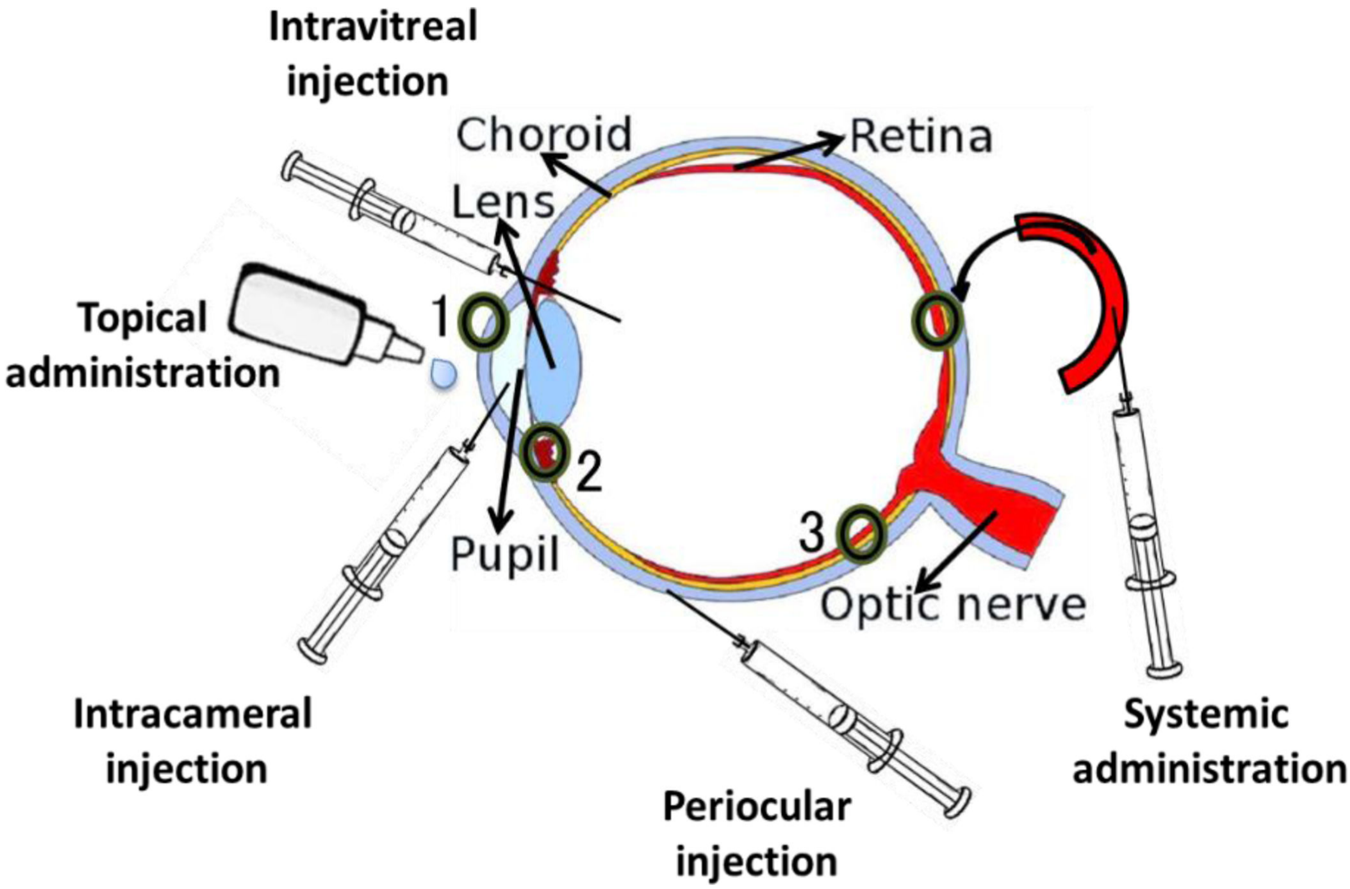

Fig. 2.
Schematic illustration of the routes and barriers to ocular drug delivery **(1)** Corneal barrier; **(2)** Blood-aqueous barrier and **(3)** Blood-retinal barrier.

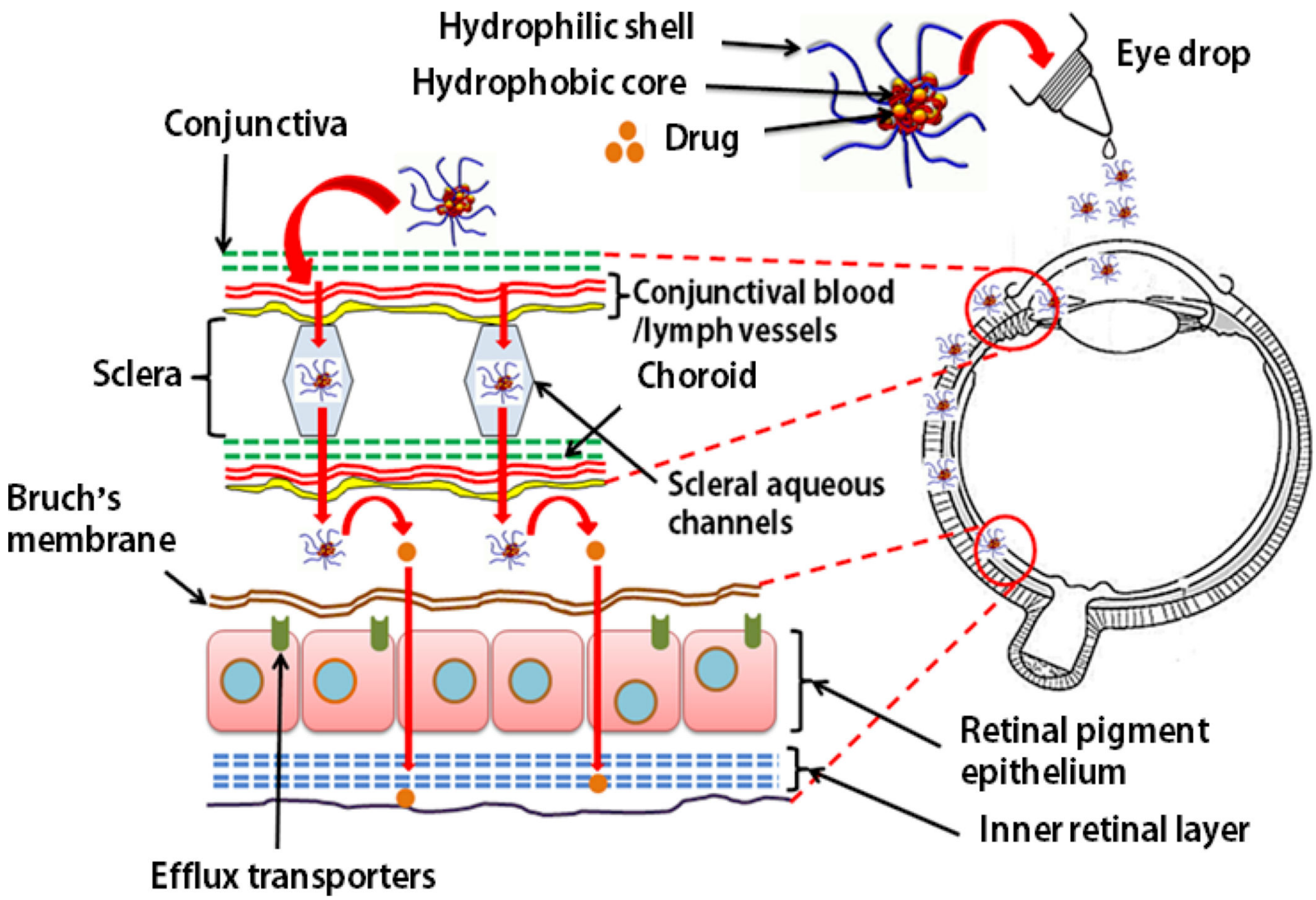

Fig. 3.
Schematic representation of polymeric micelles reaching the posterior ocular tissues via the transcleral pathway after topical application.

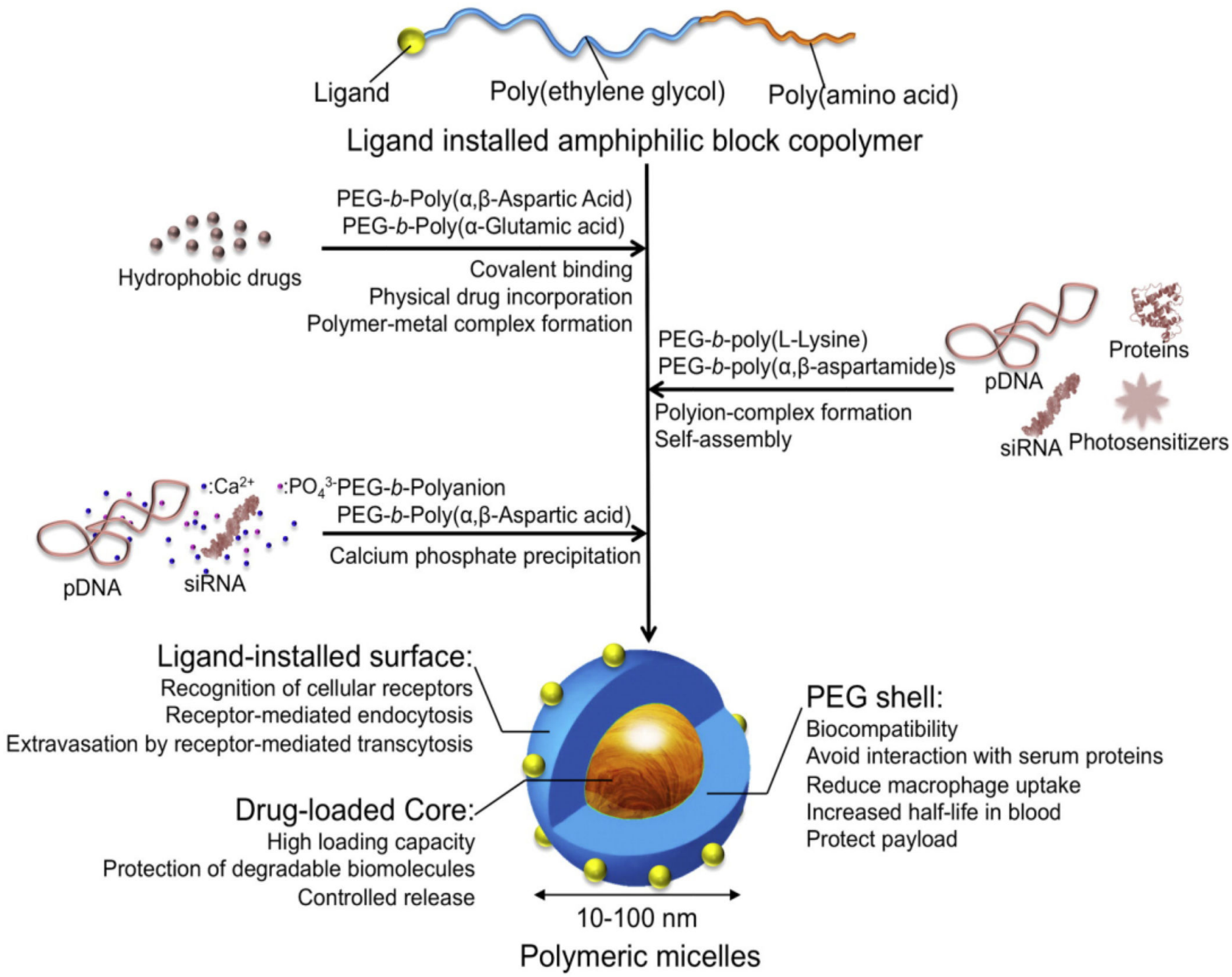

Fig. 4.

Polymeric micelles composed of PEG-b-poly(amino acid) copolymers represent a self-assembling versatile platform for encapsulating various therapeutic molecules through controlled interaction of the cargo and the core-forming segments. The relatively small size and PEG shell offers an additional advantage for interaction with biological interfaces [49]. Reprinted from with permission from Elsevier.

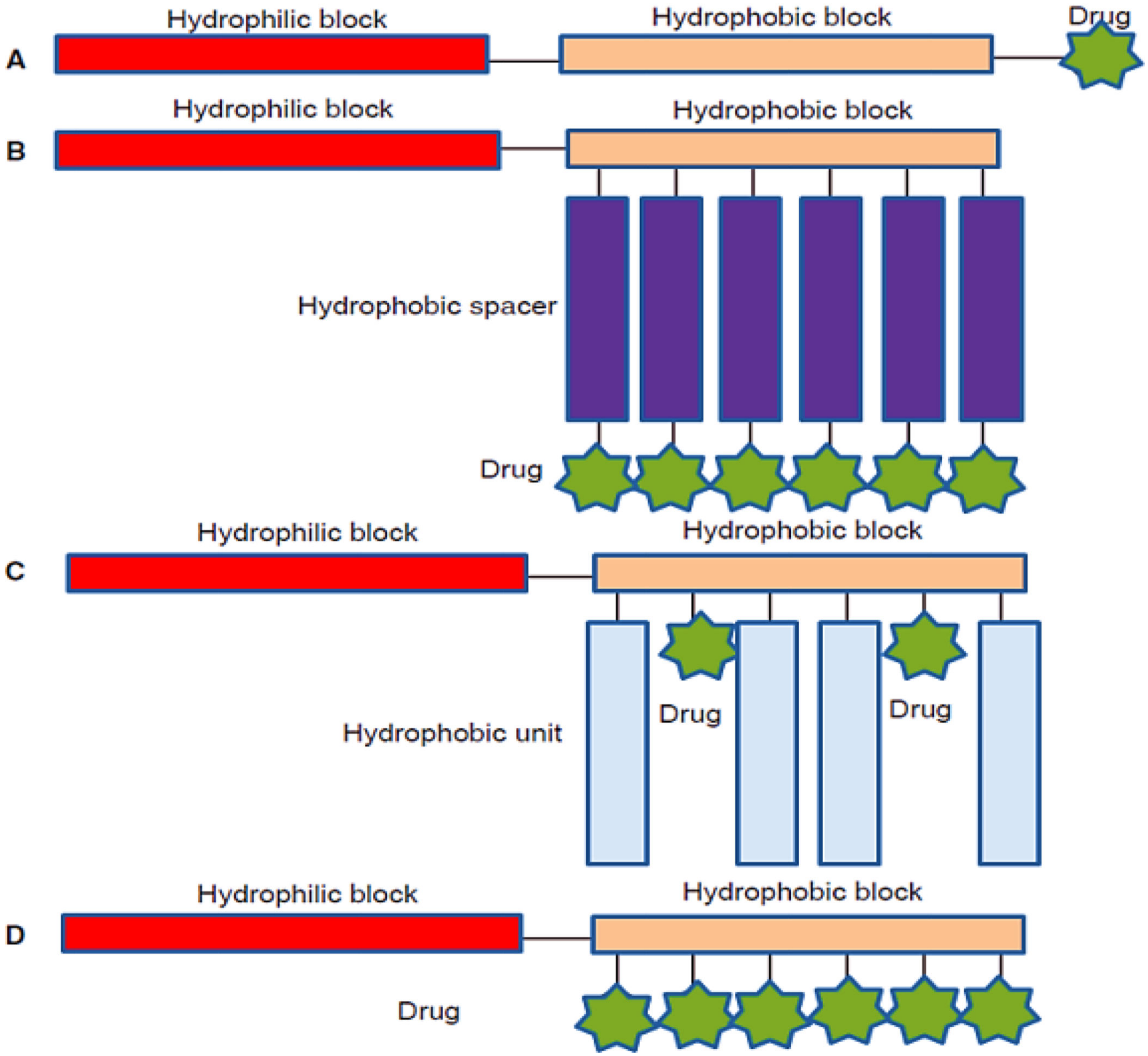

Fig. 5.

Different designs for micelle-forming drug–block copolymer conjugates. (Modified from Ref. [51]).

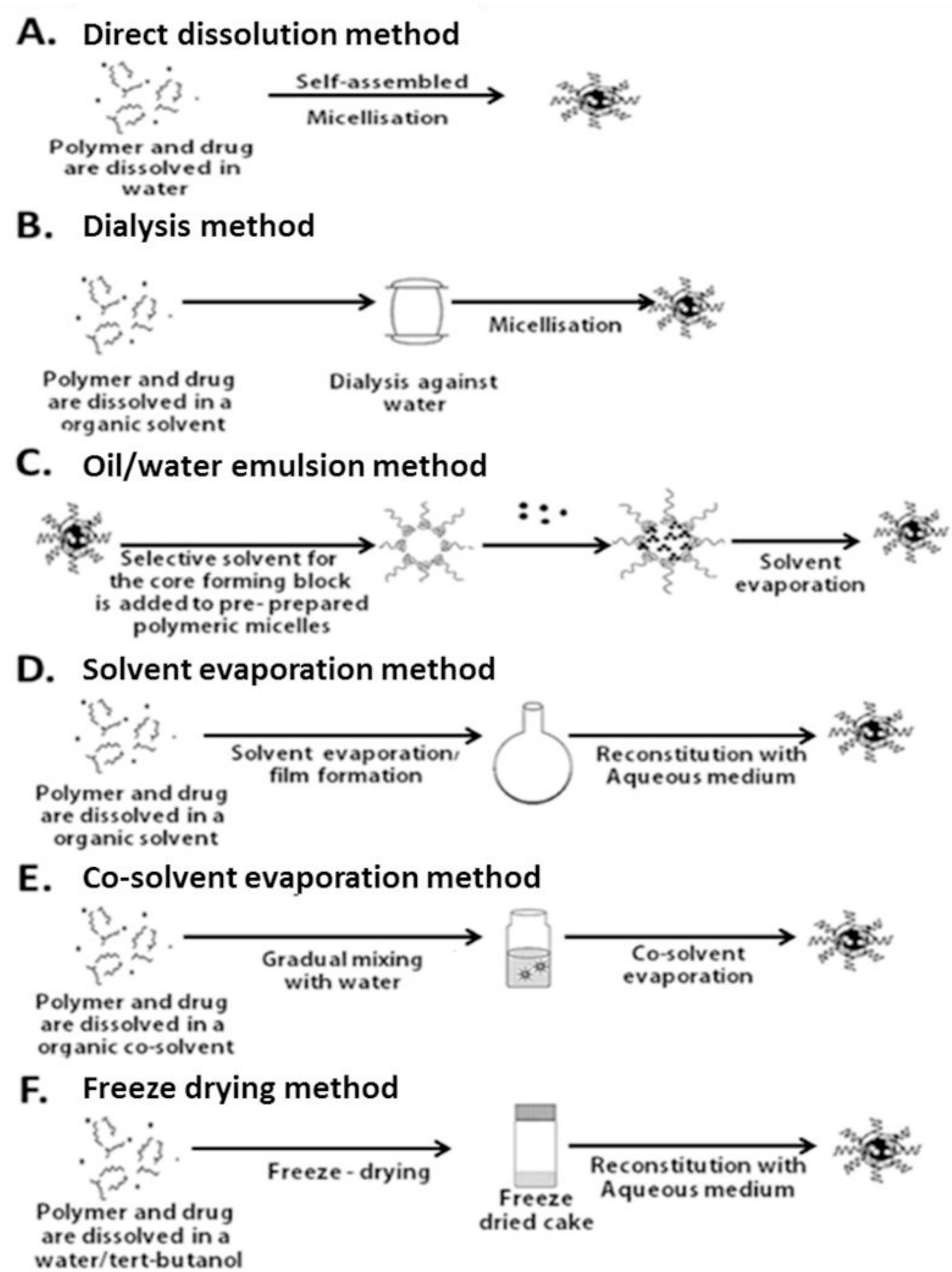

Fig. 6.

Physical methods of drug encapsulation into polymeric micelles: **(A)** direct dissolution; **(B)** dialysis; **(C)** oil-in-water emulsion; **(D)** solvent evaporation; **(E)** co-solvent evaporation; **(F)** freeze-drying (Modified from Ref. [51]).

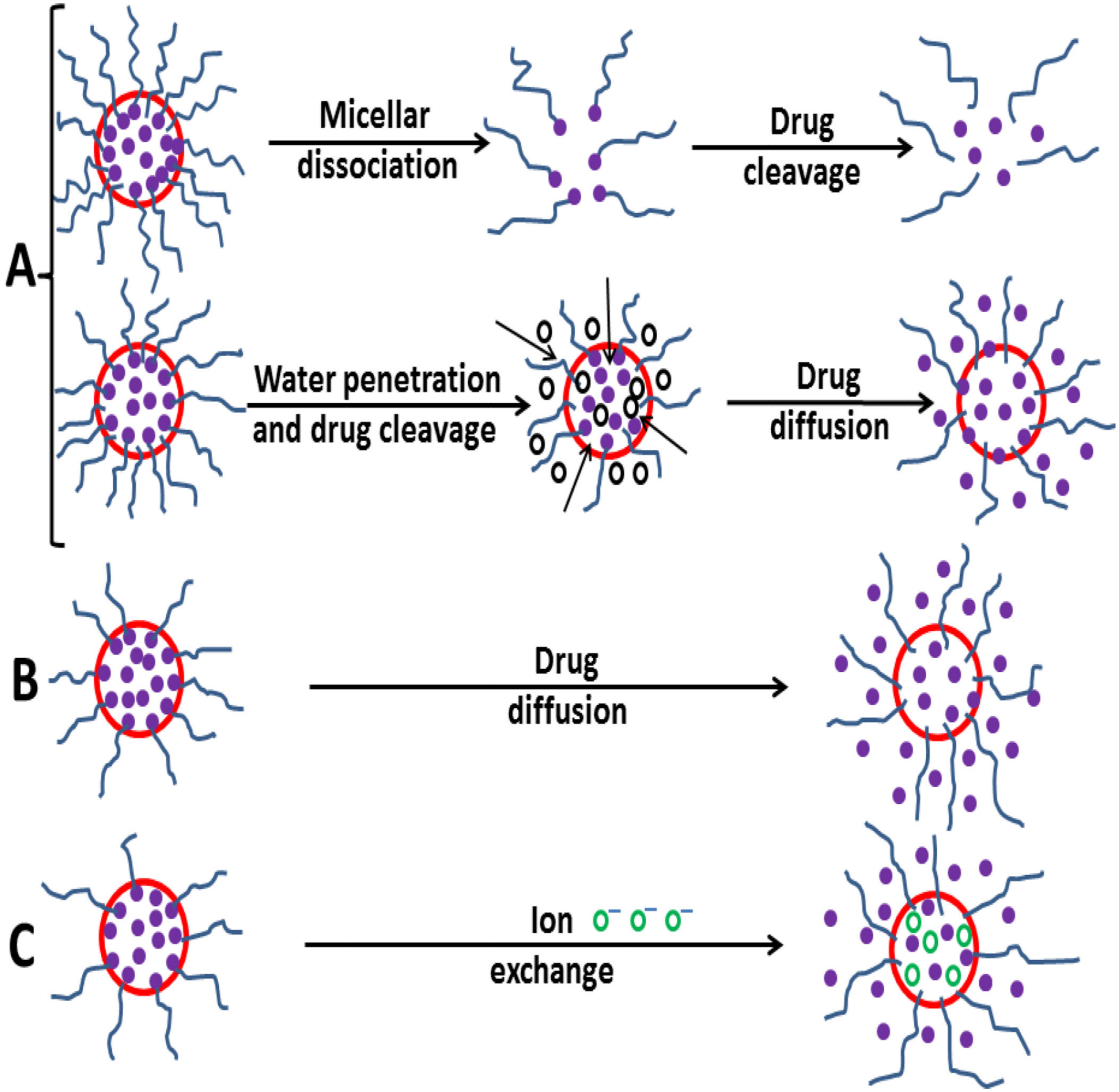

Fig. 7.

Modes of drug release from polymeric micelles. **(A)** Drug release from block copolymer-drug conjugates, **(B)** Drug release from drug encapsulated micellar carriers and **(C)** Drug release from polyion complex micelles (Modified from [51]).

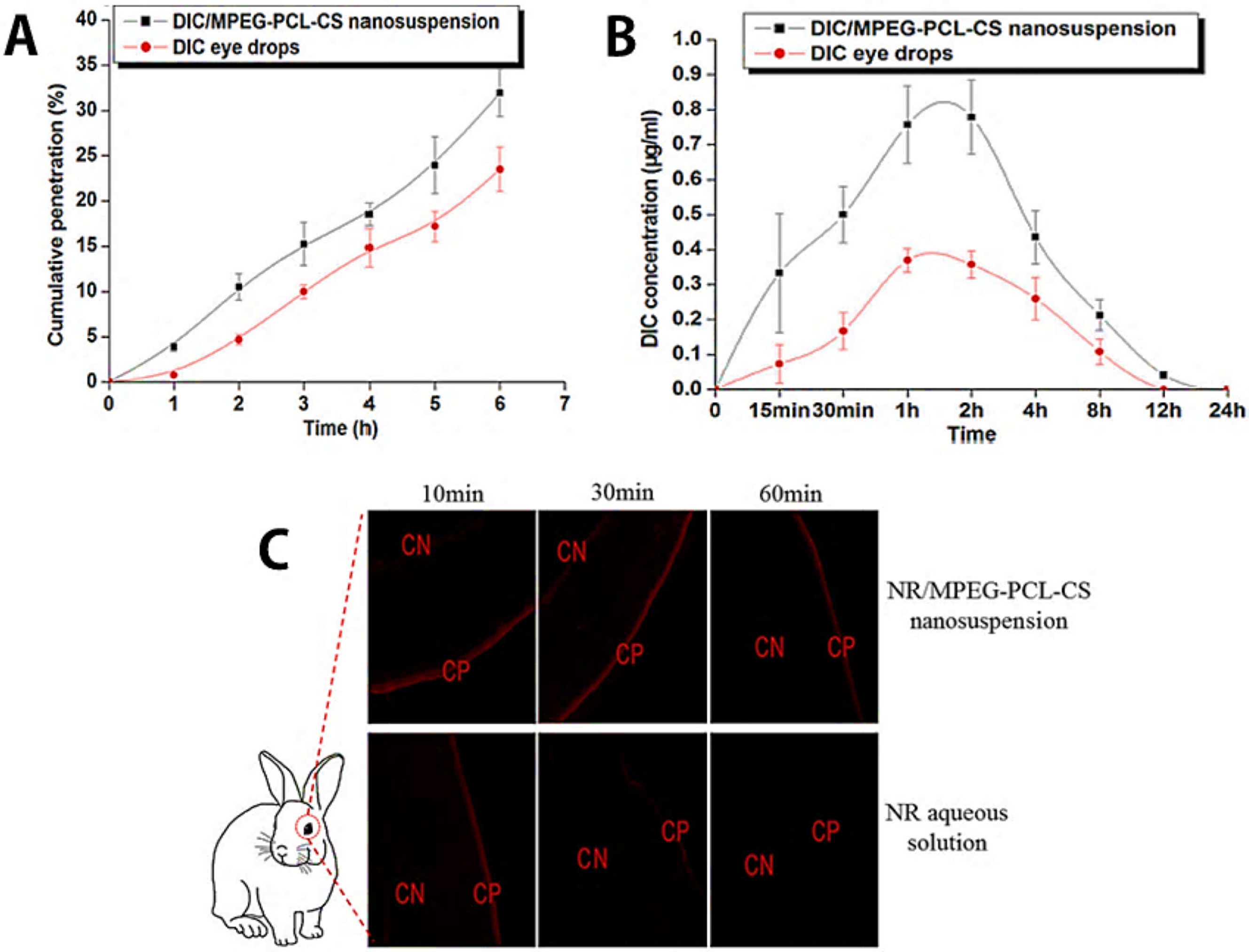

Fig. 8.

(A) 0.1% DIC commercial eye drop and 0.1% DIC/MPEG-PCL-CS nanosuspension corneal penetration profiles in-vitro. **(B)** Concentration profiles of DIC after instillation of 50 μL of 0.1% DIC commercial eye drop and 0.1% DIC/MPEG-PCL-CS nanosuspension in rabbit aqueous humor. **(C)** Fluorescence microscopy images of rabbit corneas after treatment with Nile Red (NR) aqueous solution and the NR/MPEG-PCL-CS nanosuspension. CN = corneal endothelium and CP = corneal epithelium [126]. Reprinted from with permission from Nature Publishing Group (NPG).

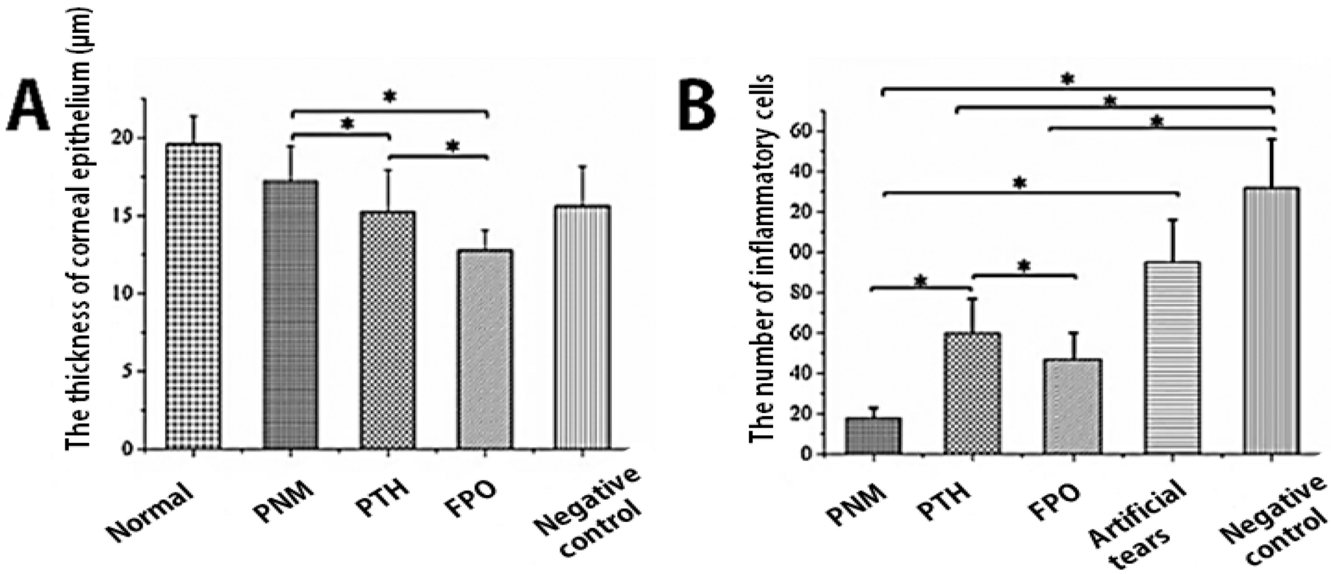

Fig. 9.

(A) Thickness of the corneal epithelium was assessed between the PNM, PTH and FPO groups. Data shown as mean ± SD. Significance was assessed between the respective groups. **(B)** Number of inflammatory cells. Data represented as mean ± SD. Significance was assessed between the PNM, PTH, FPO, artificial tears and the negative control group,*P < 0.05. PNM=pimecrolimus nanomicelles, PTH= pimecrolimus thermosensitive hydrogel, FPO= free pimecrolimus oil-based eye drop [127]. Reprinted with permission from Elsevier.

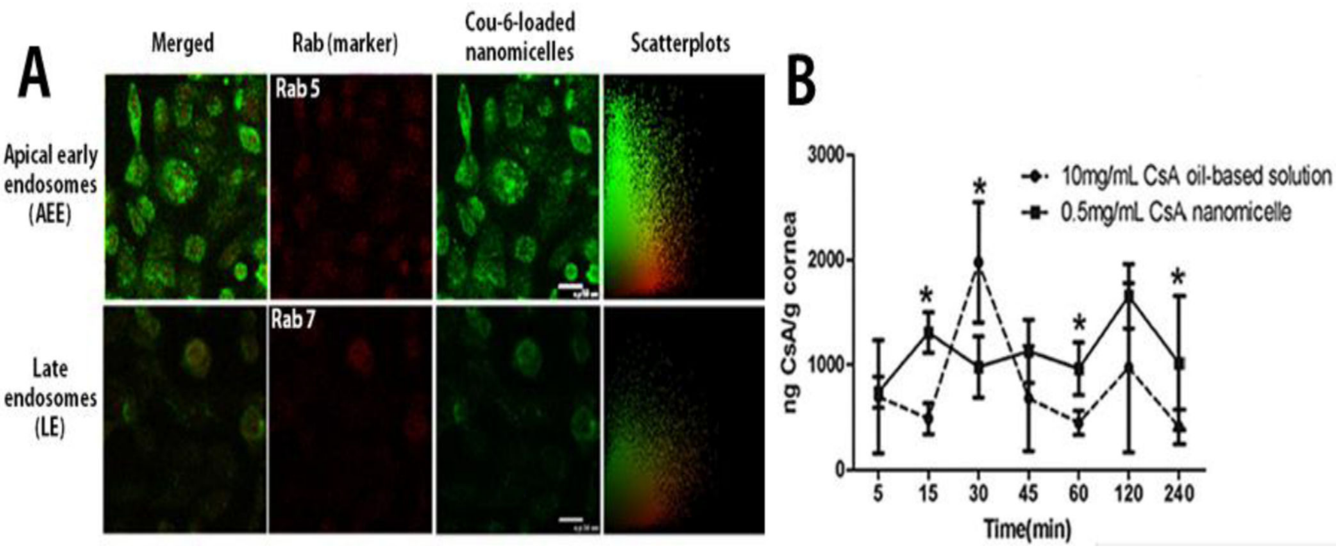

Fig. 10.

(A) Intracellular trafficking of nanomicelles in endosomes: Co-localization micrographs and scatterplots of nanomicelles with AEE and LE markers at 60 min. **(B)** CsA concentration in rabbit corneas after a single instillation (50 μL); (*$P < 0.05$ when compared to a 10 mg/mL CsA ophthalmic oil-based drop, n = 6) [130]. Reprinted with permission from Nature Publishing Group (NPG).

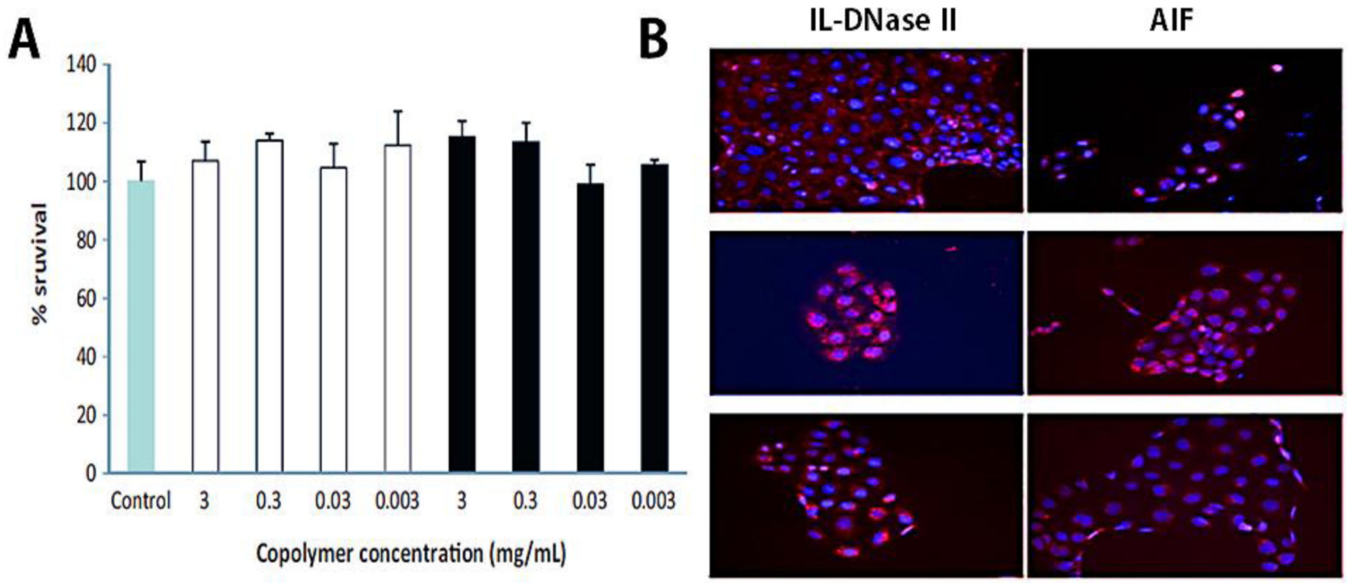

Fig. 11.

(A) Percent cell survival after 1 h of incubation with formulations. The light blue column is the control (constituted of one part 10 mM phosphate buffer with 10% sucrose and two parts of cell growth medium). White columns are the unloaded micelles and black columns are CsA loaded micelles demonstrating the biocompatibility of the formulation. **(B)** Indirect immunofluorescence of IL-DNase II and AIF after 1 h of incubation (Blue: nuclei stained with DAPI. Red: antibody under investigation (LC3)). This indicated CsA-loaded micelles did not activate the cellular mechanism that leads to caspase independent apoptosis under the applied conditions [77]. Reprinted with permission from Elsevier.

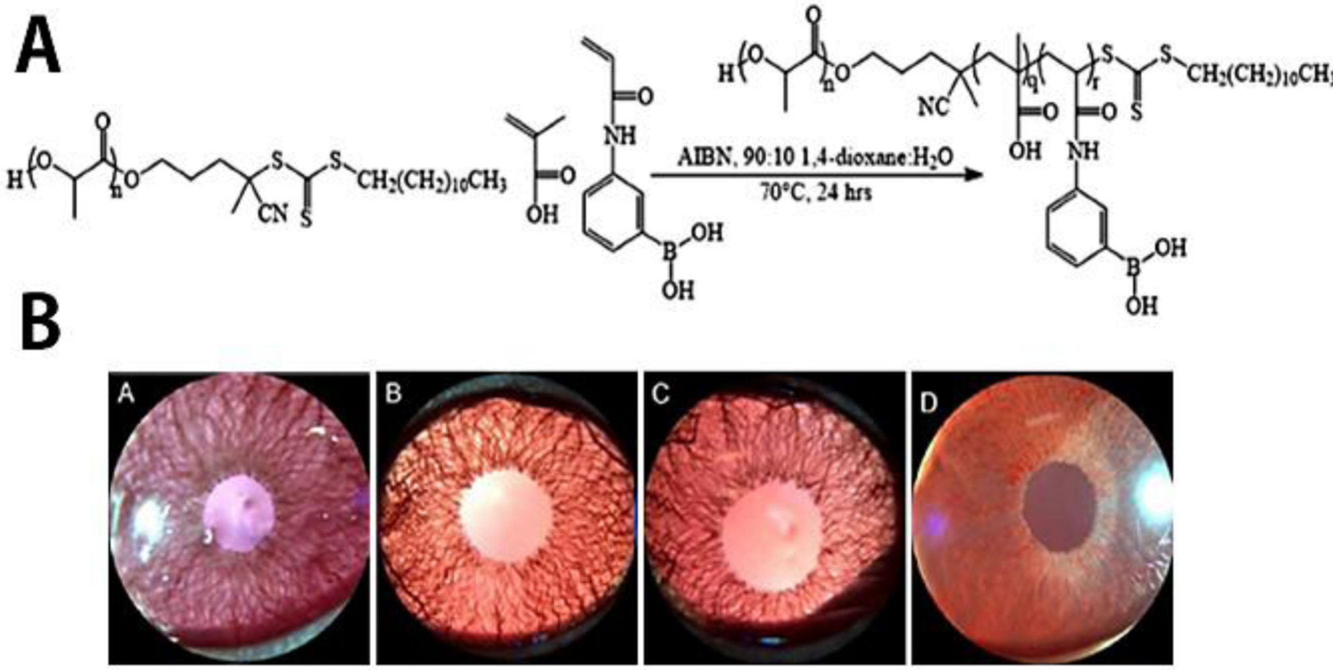

Fig. 12.
(A) Reversible addition−fragmentation chain transfer polymerization reaction mechanism utilized to synthesize the LMP block copolymers and **(B)** Slit lamp images after application of various LMP micellar formulations (A, B, C) and negative control (D) to the corneal surface [139]. Reprinted (adapted) with permission from American Chemical Society.

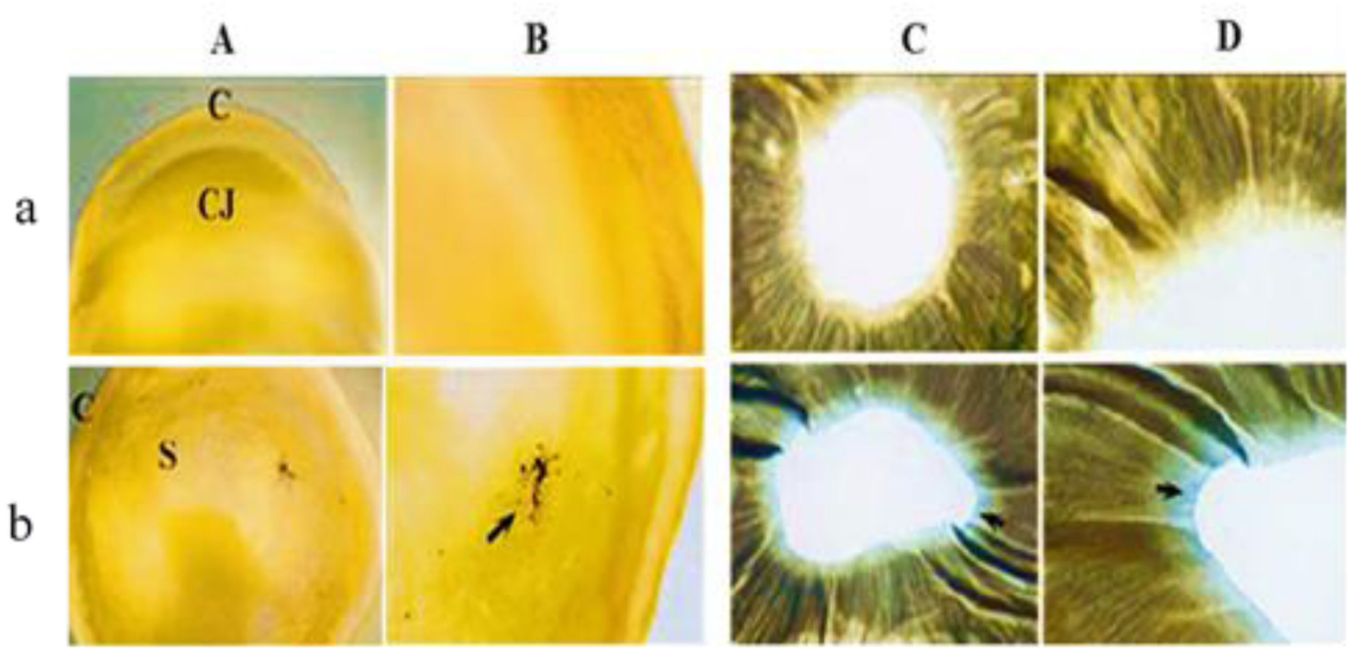

Fig. 13.

Whole-mount of eyes 2 days after topical eye-drop administration in nude mice (columns **A** and **B**) and irises of rabbits (columns **C** and **D**) with the 0.08 mL/mL plasmid/PEO-PPO-PEO polymeric micelles. **(a)** plasmid only; **(b)** plasmid/PEO-PPO-PEO polymeric micelles. The speckled blue staining (arrows in black) correspond to X-gal activity (a β-Gal substrate, which is a protein for which the plasmid encodes the lacZ gene) in the ocular tissues (C, cornea; CJ, conjunctiva; S, sclera) [142]. Reprinted with permission from Nature Publishing Group (NPG).

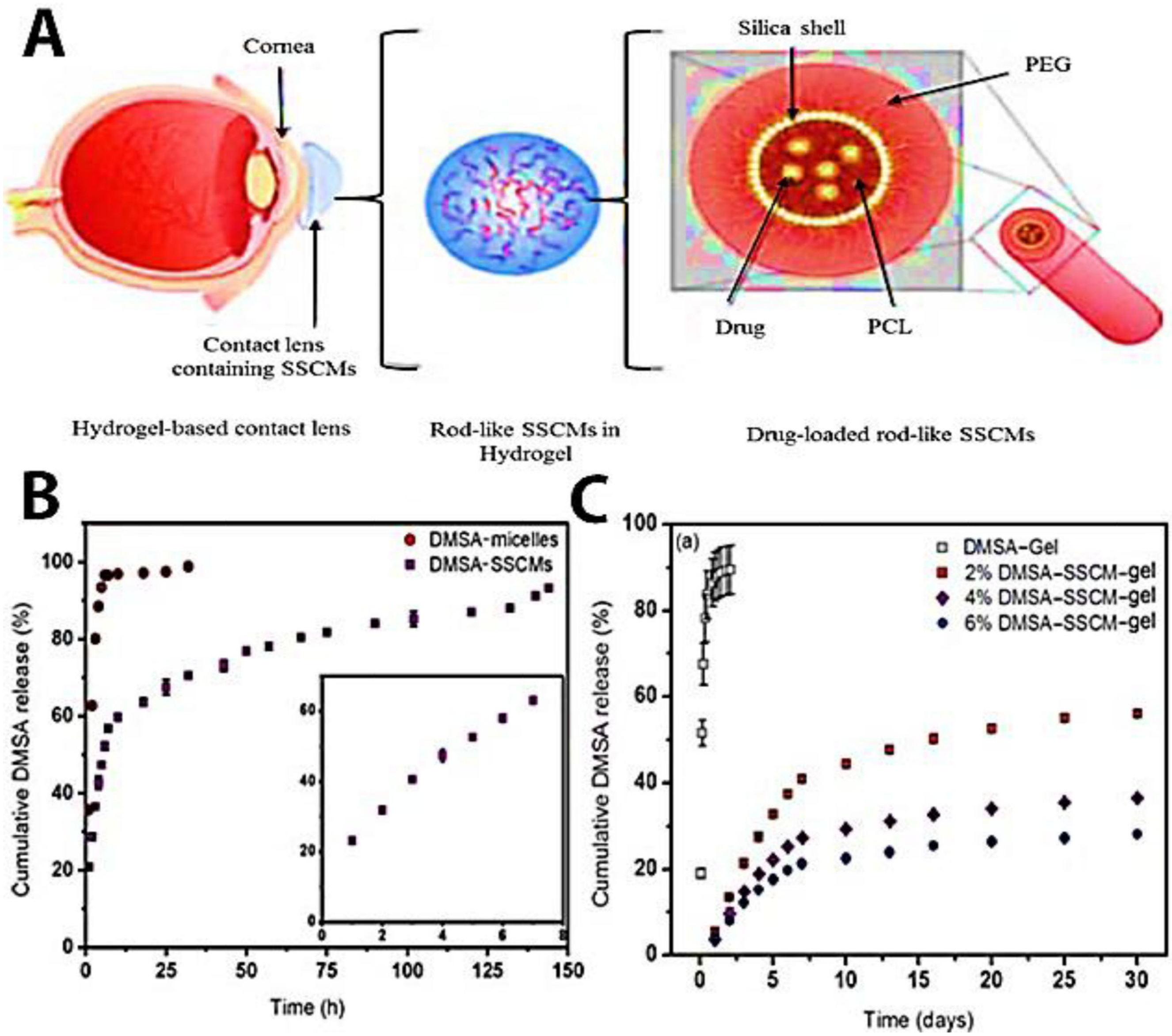

Fig. 14.

(A) Drug-loaded silica shell crosslinked micelles (SSCMs) dispersed in a hydrogel as a potential contact lens material for therapy of ocular diseases; **(B)** In vitro release of DMSA from non-shell-cross-linked PEG-b-PCL micelles and SSCMs. Inset release profile for DMSA from SSCMs during the initial 7 h of incubation and **(C)** In vitro release of DMSA from hydrogels containing varying amounts of SSCMs (i.e., 0, 2, 4, and 6%) [74]. Reprinted with permission from Elsevier.

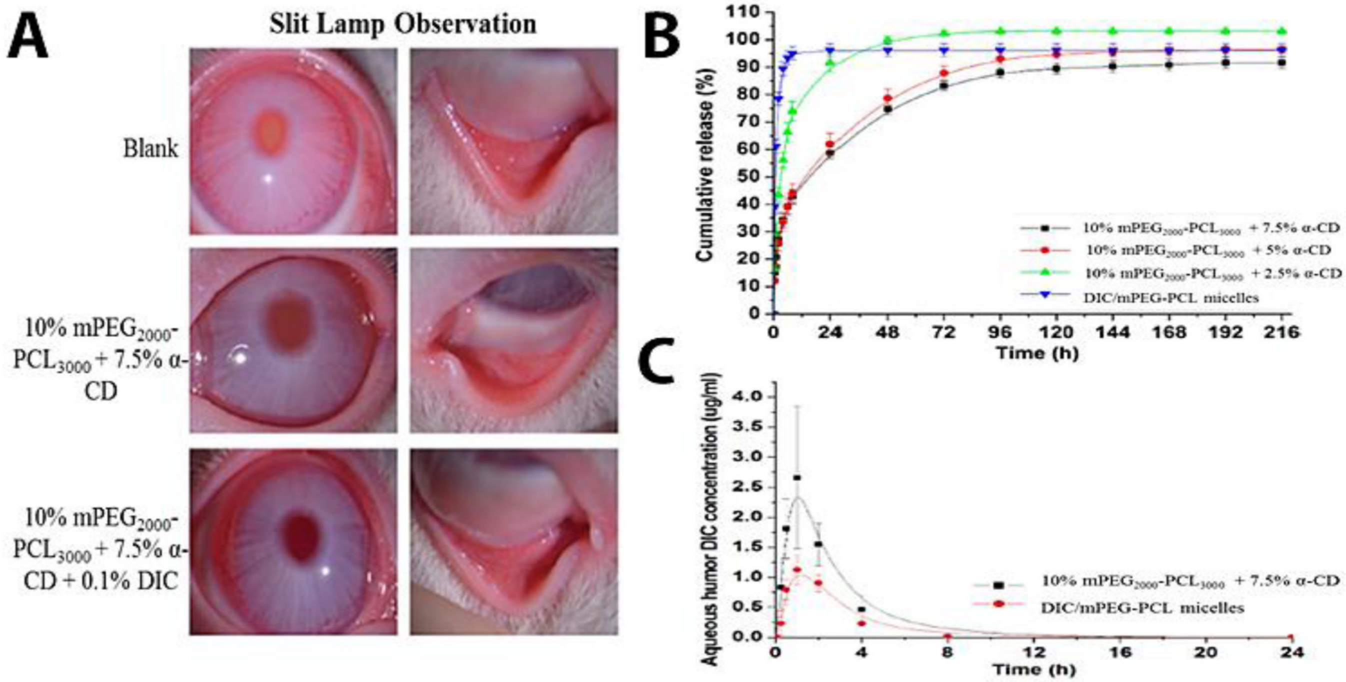

Fig. 15.

(A) Corneal surface observation 24 h after instillation of the blank and DIC-loaded micellar supramolecular hydrogels; **(B)** In vitro release profiles of DIC from the DIC/MPEG2000-PCL3000 micelles and DIC/α-CD/MPEG2000-PCL3000 micellar supramolecular hydrogels (n = 3; mean ± SD) and **(C)** Aqueous humor drug concentrations in rabbit eyes after a single instillation of 50 μL DIC/MPEG-PCL micelles and DIC loaded micellar supramolecular hydrogel (final DIC concentration: 0.1%) [146]. Reprinted (adapted) with permission from American Chemical Society.

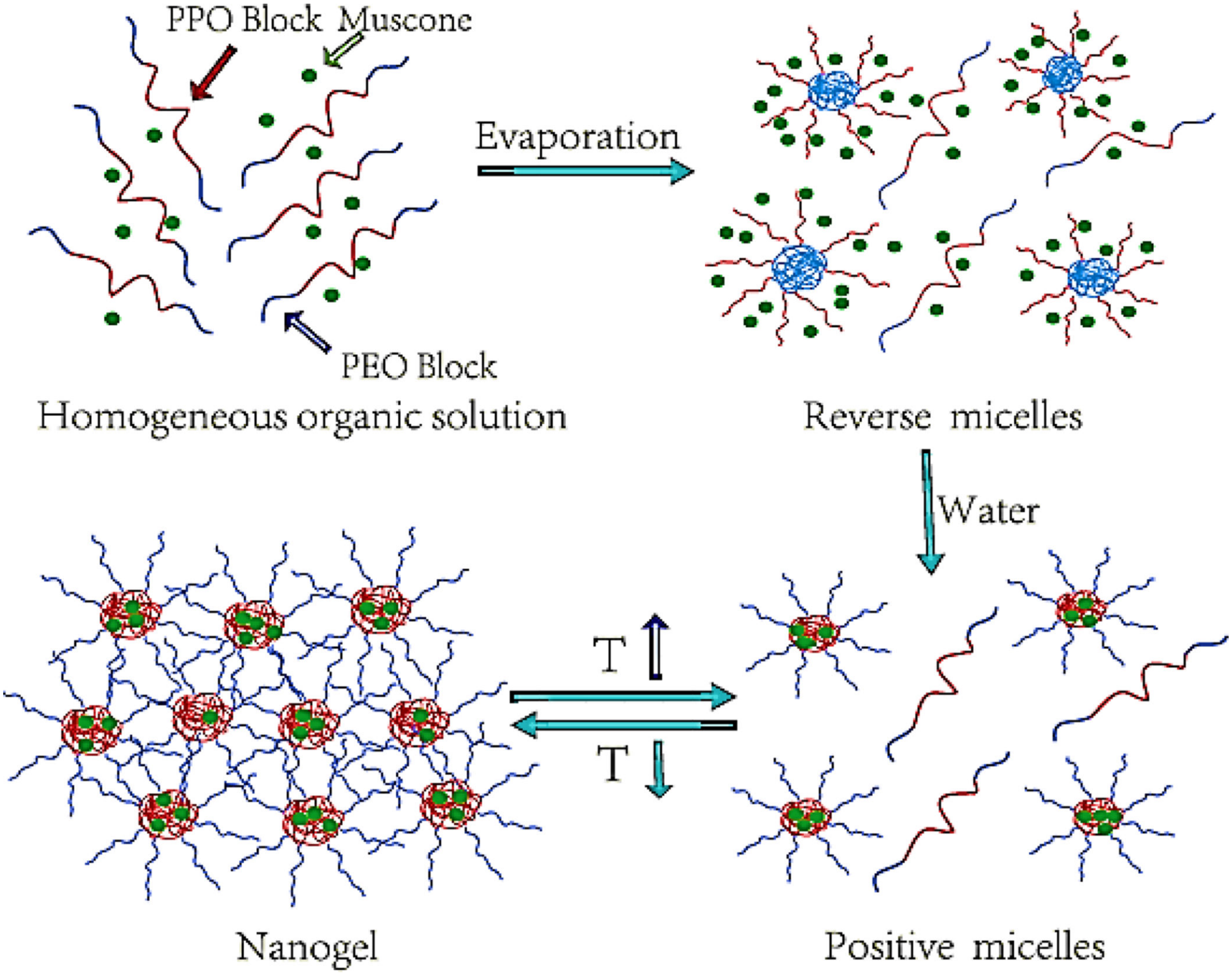

Fig. 16.

Schematic illustration of muscone thermoresponsive nanogels prepared by the RM-PM method [75]. Reprinted with permission from Elsevier.

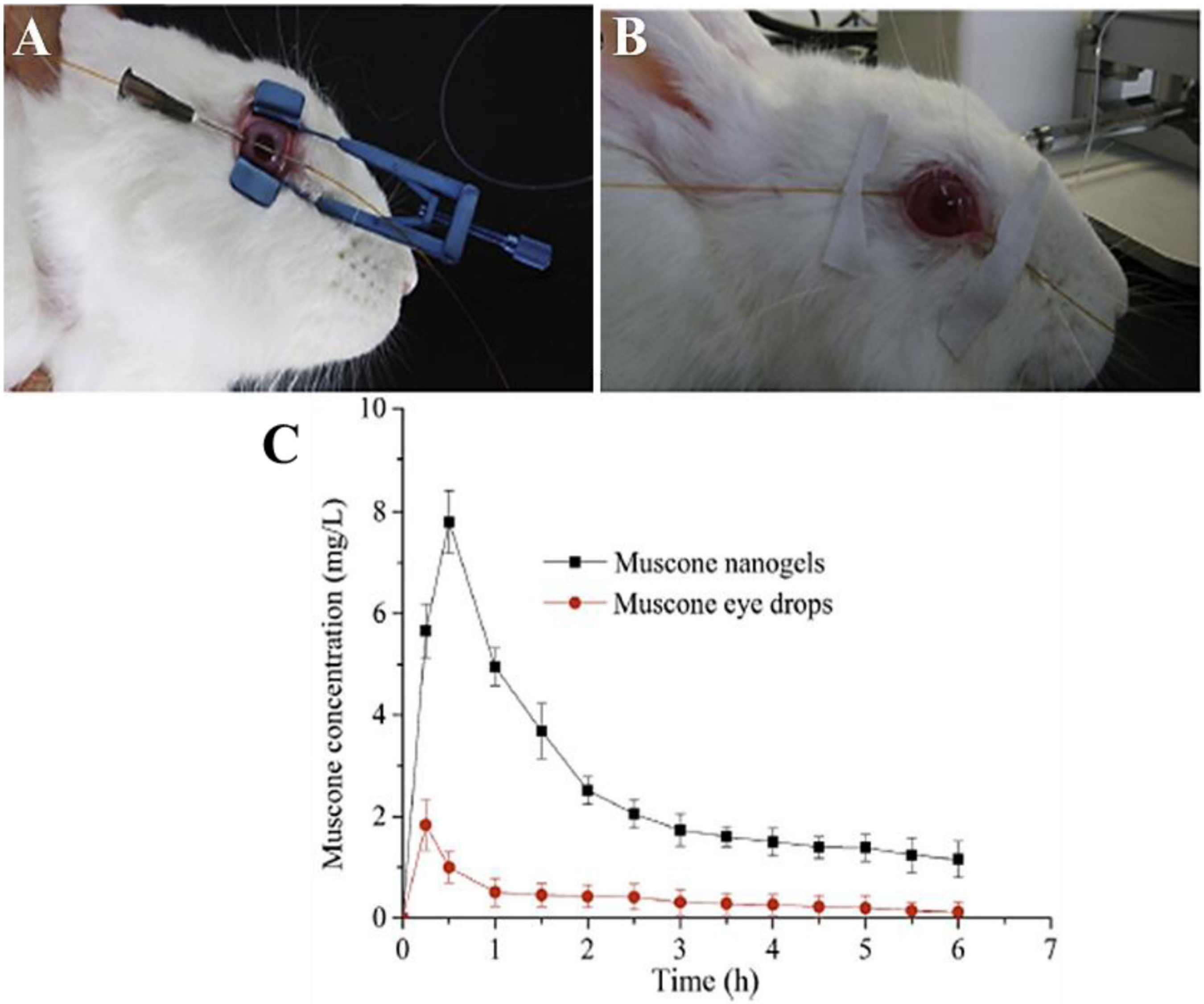

Fig. 17.
Pharmacokinetic study of muscone nanogels using the microdialysis technique **(A)** inserted across the anterior chamber; **(B)** probe implantation, **(C)** Concentration of muscone in rabbit aqueous humor at various time points after instillation of the muscone eye drops and muscone nanogels (n = 3, mean ± SD) [75]. Reprinted with permission from Elsevier.

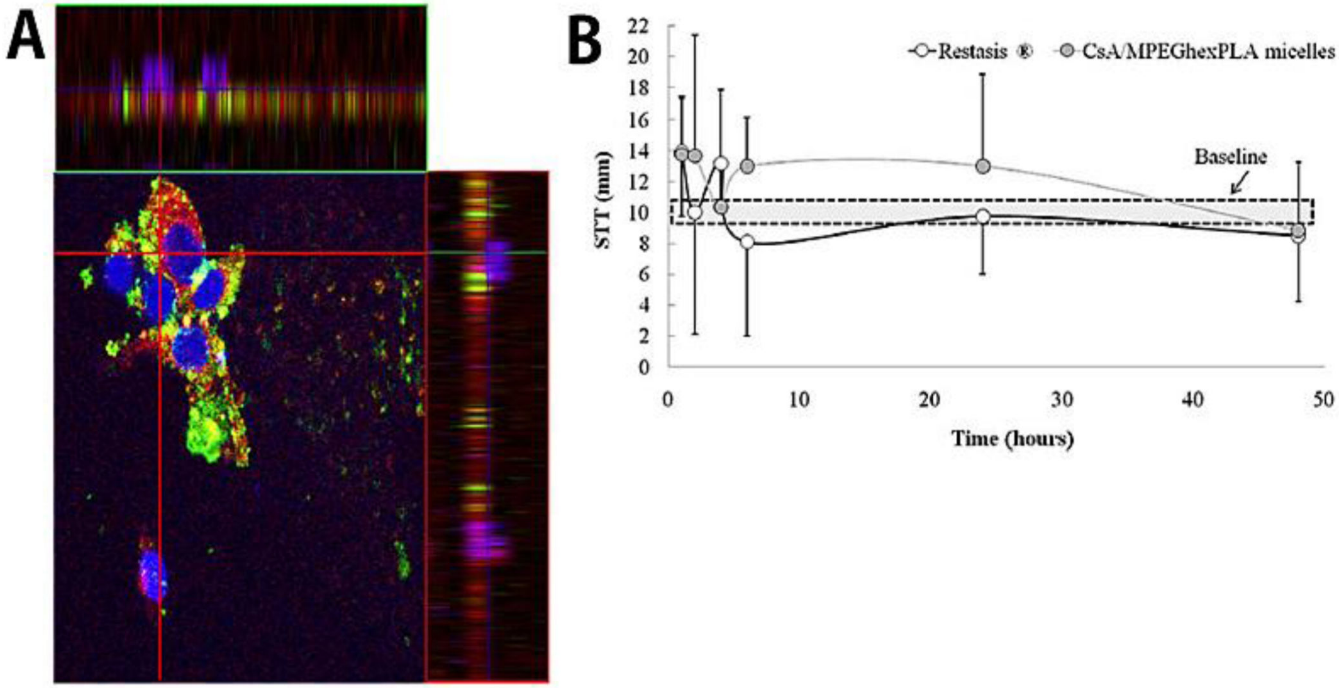

Fig. 18.

(A) Confocal microscopy images obtained from the orthogonal section of corneal cells treated with the micelle formulation after 24 h of incubation (blue fluorescence = nuclei stained with Hoechst; yellow fluorescence = DiO dye loaded fluorescent micelles; red fluorescence = micelle prepared with labeled copolymer; green fluorescence = DiO dye) and **(B)** Tear secretion mean values obtained after a single instillation of the micelle formulation or Restasis (n=6). The area with the dashed line indicates the initial tear production before instillation. Reprinted from [147].

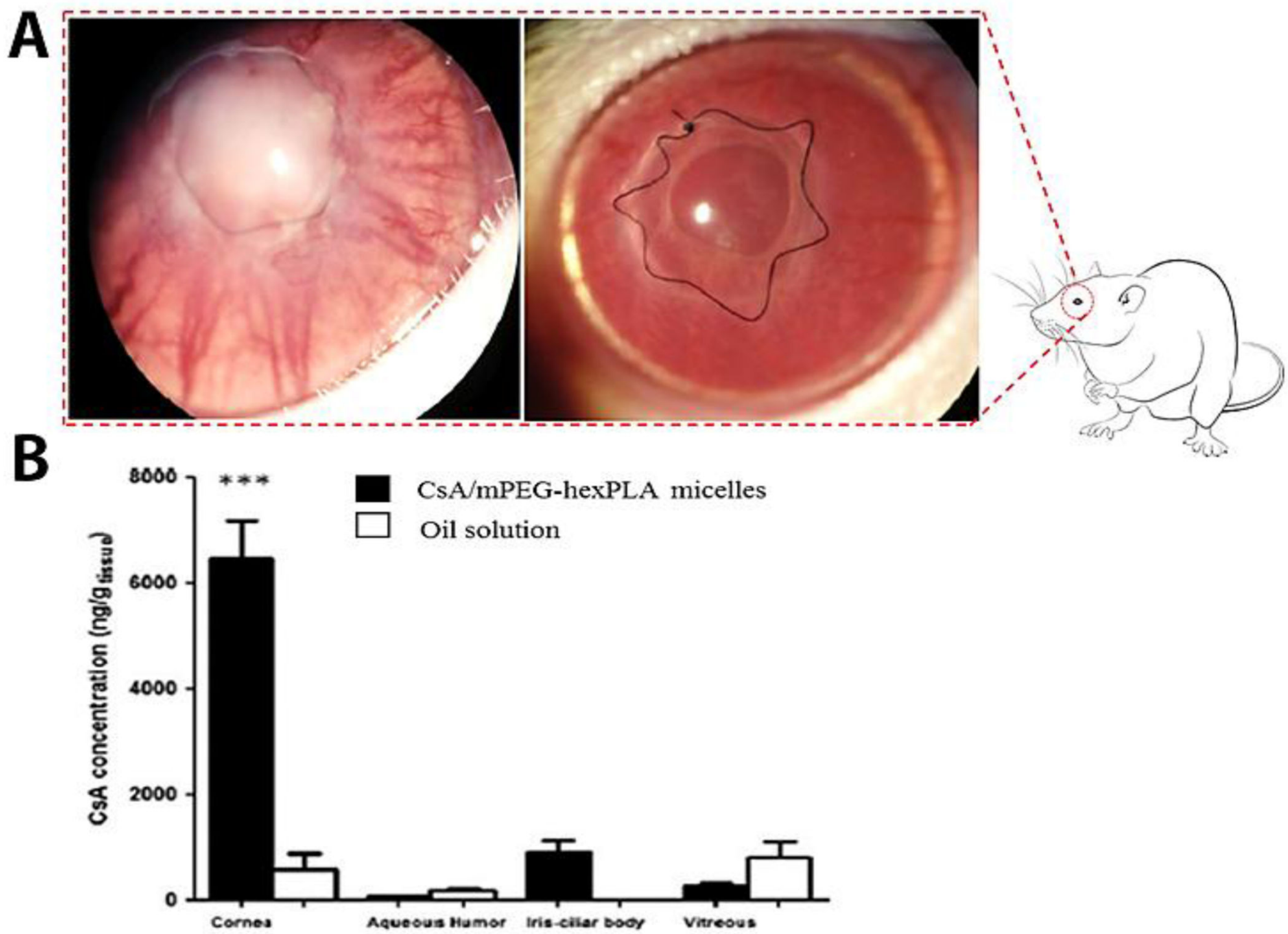

Fig. 19.

(A) Representative images of transplanted corneas at the end of the study after penetrating keratoplasty and 14 days of topical treatment. Left: a rejected graft from the group treated with a physiological saline solution; Right: an accepted clear graft from the group treated with the CsA micelle formulation and **(B)** Ocular distribution of CsA/MPEG-hexPLA micelles and oil solution after 5 days with five instillations per day. *** $p < 0.001$ [39]. Reprinted with permission from Elsevier.

Table 1

Polymers used in the development of polymeric micelles for ocular drug delivery.

Drug	Polymer(s)	Ref.
Ciprofloxacin	Pluronic F127	[69]
Itraconazole	Poloxamer 407 (Pluronic F127) Poloxamer 188 (Pluronic F68)	[70]
Diclofenac	methoxy poly(ethylene glycol)–poly(ε-caprolactone) (MPEG–PCL)	[71]
Fluconazole	poly(butylene oxide)-poly(ethylene oxide)-poly(butylene oxide)	[72]
Dexamethasone alcohol	Polyhydroxyethylaspartamide (PHEA)	[73]
Dexamethasone acetate	Methoxy(polyethylene glycol)-block-polycaprolactone (MePEG-b-PCL)	[74]
Cyclosporine A (CsA)	Methoxy poly(ethylene) glycol (MPEG)	[39]
Muscone	Poly(ethylene oxide)–poly(propylene oxide) (PEO-PPO-PEO) block copolymers	[75]
Cidofovir	Hexadecyloxypropyl	[76]
Cyclosporine A	Methoxy poly(ethylene glycol)-hexylsubstituted poly(lactide)	[77]
Curcumin	Pluronic P123 (P123)/D-a-tocopheryl polyethylene glycolsuccinate (TPGS)	[78]
Ketorolac	N-isopropylacrylamide (NIPAAM)	[79]
Lornoxicam	Tetronic® 701 (T701) Synperonic® PE/F127 (F127), Synperonic® PE/P84 (P84)	[80]
α-tocopherol (TOC)	Poly(propylene oxide) (PPO) and poly(ethylene oxide) (PEO)	[81]
Carbamazepine	PEO–PPO–PEO	[82]
Dexamethasone	Polyoxyethylated nonionic surfactant Pluronic® F127 (F127) cationic polyelectrolyte chitosan (CH)	[83]
Dexamethasone triamcinolone acetonide and β-estradiol (E2β)	Egg lecithin (LE)	[84]
Dasatinib	PEG-b-PCL	[85]
Anti-apoptotic gene (bcl-X_L)	Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO)	[86]

Table 2

Micelle-loaded *in-situ* gelling systems for ocular drug delivery

Polymer		Drug	Observation	Ref
Micelle	**Hydrogel**			
β-CD/PAA/PEG	MPC HEMA	Orfloxacin	Micelle size decreased with an increase in β-CD content, but increased with an increased PAA/PEG concentration. Micelle loaded system released drug in a sustained manner for more than 24 h.	[145]
Pluronic F127 and Pluronic F68	Carbopol 934P	Itraconazole	Compared to Itral® eye drops and a pure drug suspension, micelle-loaded gels results into enhanced ex vivo transcorneal penetration (41.45 ± 0.87%) with sustained drug release.	[70]
PEG-b-PCL	pHEMA	Dexamethas one	Micelle/pHEMA hydrogels provided controlled drug delivery for more than 14 days.	[20]
MPEG-block polymers	MPEG-PCL with α-CD	Diclofenac	Hydrogels showed low cytotoxicity towards L929 and HCEC cells. In-vivo studies showed no irritation in rabbit eyes with extended corneal retention compared to a plain micellar formulation.	[146]
$MePEG_{2k}$-b-PCL_{2k}	pHEMA	Dexamethas one acetate	Rod shaped silica shell cross-linked micelle loaded hydrogel showed in-vitro drug release for 30 days while maintaining optical transparency.	[74]
Pluronic P123 and TPGS	Gellan gum	Curcumin	Non-irritant and biocompatible formulation. Ex-vivo studies showed enhance transcorneal penetration using non-ionic surfactants.	[78]

β-CD: Beta-cyclodextrin; PAA: Polyacrylic acid; PEG: Polyethylene glycol; MPC: 2-methacryloyloxyethyl phosphorylcholine; HEMA: Hydroxyethyl methacrylate; PCL: poly(ε-caprolactone) pHEMA: Poly(2-hydroxyethyl methacrylate; MPEG: Monomethoxy polyethylene glycol; α-CD: Alpha-cyclodextrin; MePEG-*b*-PCL: Methoxy poly(ethylene glycol)-block- poly(ε-caprolactone) and TPGS: D-a-tocopheryl polyethylene glycolsuccinate.

Table 3

Summary of recent patented polymeric micelle formulations for ophthalmic applications currently under preclinical evaluation.

Patent number	Year	Formulation characteristics	Ref.
US8980839 B2	2015	Aqueous nanomicellar ophthalmic solution comprising cyclosporine, a polyoxyl lipid or fatty acid and a polyalkoxylated alcohol	[148]
WO2015041520 A1	2015	Peptide-based self-assembling micelles	[149]
US 9017725 B2	2015	Nanomicelles consisting of dexamethasone, vitamin E TPGS and octoxynol-40.	[150]
CN104644550 A	2015	A curcumin micelle drops, comprising curcumin, chitosan micelle drug carriers, surface active agents, surfactants and pharmaceutical purified water.	[130]
US 8697098 B2	2014	Prolamine protein conjugated to a polymer, such as a polyethylene glycol (PEG) chain, with conjugates used to prepare micelle assemblies.	[151]
US8470371 B2	2013	A polymeric micellar aggregate having a mean particle size between 20 and 500 nm formed from an amphiphilic carbohydrate polymer.	[152]
US 20120225834 A1	2012	Ocular iontophoresis consisting of micelles comprising one or more charged surfactants and one or more bioactive agents.	[153]
US 20120294945 A1	2012	Micelle consisting of hyaluronic acid and a core region comprising a water-insoluble peptide with a terminal amine group, wherein the water-insoluble peptide is bound to hyaluronic acid or its pharmaceutically acceptable salt.	[154]
WO2010144194	2010	Mixed nanomicellar formulations (vitamin E, TPGS, octoxynol-40) of water insoluble drugs.	[155]
WO 2011041377 A1	2010	Ocular iontophoresis of charged nanomicelles consisting of alkyltrimethylammonium halide, alkyldimethylammonium halide, alkylmethylammonium halide, alkylethyldimethylammonium halide, alkyldimethylbenzylammonium halide, alkylpyridinium halide, and alkylimidazolium halide, or a mixture of two or more.	[156]
WO2008004978 A1	2008	A block copolymer comprising at least a first block and a second block, wherein the first block comprises a range of temperature-sensitive monomeric units, a range of hydrophilic monomeric units and a range of targeting monomeric units. The second block comprises a range of hydrophobic monomeric units and at least one pH-sensitive moiety.	[157]
WO2008017839 A1	2008	Polymeric micellar clusters formed from amphiphilic carbohydrate polymer.	[158]
US 20060110356 A1	2006	Micelles with a hydrophilic polymer chain of polyoxyethylene or polyethylene glycol and a hydrophobic polymer chain of polylactone.	[159]
EP1609465 A1	2005	Polymer micelle of a block copolymer wherein the hydrophilic polymer chain is polyoxyethylene or polyethylene glycol. The charged polymer chain is a polyamine, a polycarboxylic acid or a polypeptide.	[160]
CA 2520525 A1	2004	Polymer micelle of a block copolymer comprising a hydrophilic polymer chain (polyoxyethylene or polyethylene glycol) and charged polymer chain as a core (polypeptide)	[161]
US6579519 B2	2003	Micelle solution of block random copolymers of the general formula (X 揦)$_{m}$, wherein X is a monomer selected from the vinyl group of compounds, Y is a monomer which will provide thermo-sensitivity of the co-polymer having a general formula R_1-R_2N-(C=O)-CH=CH_2. Z is a monomer selected from acrylate based monomers which will provide mucoadhesiveness and pH-sensitivity.	[162]
US5955509 A	1999	Poly(vinyl N-heterocycle)-block-poly(alkylene oxide) copolymer based micelles	[163]
US 5766580 A	1998	Micelle comprising ethylene oxide-propylene oxide-ethylene oxide block copolymer.	[164]