Ophthalmic gels: past, present and future

1 Introduction

The organ of vision "the eye" is one of the most confined, yet complicated, organs of the body. The eye can be divided anatomically into precorneal area, anterior and posterior segments (Figure 1). Each region is susceptible to a number of diseases that may require treatment via different routes of administration, using various modalities (Figure 1) [1].

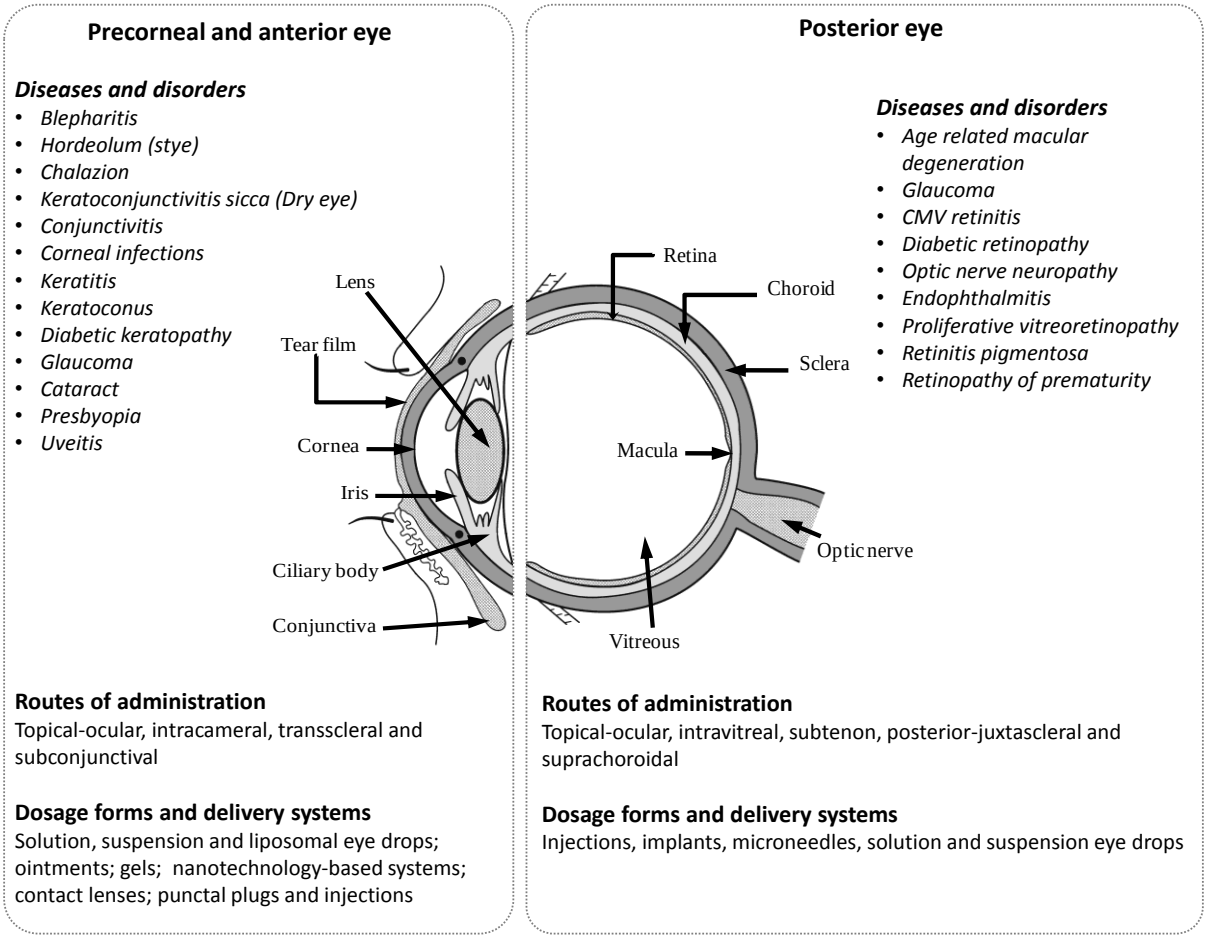

Figure 1 Cross section of human eye showing the eye anatomy along with a list of common eye diseases, routes of drug administration, ophthalmic dosage form and delivery systems

Static, dynamic and metabolic barriers of the eye make topical drug administration rather challenging. Static barriers are the physical stumbling blocks that drugs must overcome to exert a pharmacological effect on the eye; they include the conjunctiva, sclera and cornea.

These barriers pose a challenge due to their different characteristic features (thickness, polarity, collagen content, hydrophilicity, surface charge etc). The conjunctiva has always been perceived as a major site of loss through which topically administered drugs end up in the systemic circulation rather than acting on the eye. The sclera has been shown to be

more permeable to hydrophilic drugs; on other hand the corneal epithelium is more in favour of lipophilic drugs [2]. Dynamic barriers are best represented by the clearance of the drug from the eye via conjunctival blood vessels, lymph vessels and nasolacrimal drainage. The human eye contains metabolic enzymes, such as esterase and carbonic anhydrase, that can deactivate drugs [3]. Collectively, these mechanisms lead to reduced precorneal residence time, compromised ocular absorption and bioavailability, necessitating frequent drug administration, often leading to poor patient compliance.

Conventional ophthalmic dosage forms such as eye drops (formulated as solutions or suspensions) and ointments are preferred to deliver drugs to the ocular surface and the anterior eye segment. Their relative ease of use, non-invasiveness, low production cost and ease of manufacturing provide distinct advantages [4, 5]. However, aqueous eye solutions suffer very short contact time with the ocular surface due to rapid nasolacrimal drainage, leading to poor ocular bioavailability. Suspensions often give rise to unpredictable and variable ocular bioavailability; whereas ointments compromise visibility and lack patient acceptability, may induce reflex blinking and can result in the closure of the puncta and canaliculi [6].

The major problem with topical ocular therapeutics is the attainment of an optimal drug concentration at the target site. To overcome this, formulation scientists have researched and developed various drug delivery systems with enhanced ocular residence time [1, 7, 8]. Examples of such systems are medical gels, which have attracted considerable interest since the first produced soft contact lenses and implantable material prepared from hydroxyethyl methacrylate polymer in the 1960's [9]. More recently, *in situ* polymeric gelling systems (also called gel-forming systems) are being investigated for enhanced ocular drug delivery. These are prepared as liquid dosage forms which undergo phase transition on the ocular surface or conjunctival *cul-de-sac* to form a viscoelastic gel in response to an environmental stimulus, following topical application [10]. The environmental stimuli can be sub-divided into three main categories; physical (light and temperature) [11, 12], chemical (pH, redox potential, electrolyte- or molecular triggered response) [13, 14] and biological (biomolecules such as enzymes), each with a different mechanism for gel formation (outlined in Section 2) [15, 16]. These triggers can be modified to control gel formation, ocular residence time and drug release. *In situ* polymeric gelling systems have gained considerable interest as they do not require organic solvents or copolymerisation agents to trigger gel formation.

2 Ophthalmic gelling systems: polymers and mechanisms

The polymers used in preformed ophthalmic gels could be natural or semi-synthetic. Those used to form *in situ* gelling systems can be categorised, according to the environmental stimuli that triggers their conversion from sol to gel, as temperature-responsive (thermoresponsive), pH or ion-responsive gelling systems.

2.1 Thermoresponsive polymers

Thermoresponsive polymeric gelling systems are liquid at room temperature (20-25°C) and undergo a phase change, to form a gel, at 33.8°C on the ocular surface [17]. The temperature-dependent phase transition occurs when the thermoresponsive polymer in the solution becomes insoluble above or below a specific temperature, known as the lower critical solution temperature (LCST) and the upper critical solution temperature (UCST), respectively. Thermoresponsive systems which form above the LCSTs are often used in modified drug release applications, especially for protein delivery [18, 19].

2.1.1 Mechanism of gel formation

Thermoresponsive systems which form above LCSTs start as transparent, homogenous, free-flowing, polymeric solutions at temperatures below the LCST and become cloudy upon reaching the LCST. The cloudiness of the solution results from the collapse of the polymeric chains followed by aggregation and subsequently increased light scattering of the solution. Phase separation occurs past the LCST, dividing the solution into a gel-phase and a solvent phase, commonly water [20]. This is primarily due to an entropy effect, favouring phase separation which occurs upon temperature increase [21]. There are multiple variables that can be fine-tuned to obtain the desired LCST. Deen [22] has reported a change of the LCST of poly(*N*-acryloyl-*N*'-ethyl piperazine-co-methyl methacrylate) after modifying the monomer composition, pH and the addition of salts or cationic surfactants.

2.1.2 Thermoresponsive polymers of clinical potential

Thermoresponsive polymers which have extensive ophthalmic applications can be broadly classified into poly(*N*-isopropylacrilamide) (PNIPAM), poloxamers and cellulose derivatives. These polymers are used as single or combination systems. PNIPAM is the most widely used thermoresponsive polymer and has an LCST of 32°C, above which a phase transition occurs transforming the water-soluble liquid to a hydrophobic gel [21]. This type of thermoresponsive polymer has multiple applications including tissue engineering [23, 24] and surface modification for biological applications (e.g. thermoresponsive control of cell attachment and detachment [25]). For ophthalmic applications, PNIPAM has been used as a platform for controlled drug delivery [26] and has been reported to reduce intraocular pressure following *in vivo* topical [26, 27] or intravitreal [28] administration. Furthermore, the use of linear PNIPAAm crosslinked with nanoparticles as a mixture has shown greater intraocular pressure-lowering effect relative to a linear PNIPAAm system [26]. Other studies have investigated preparation and characterisation of PNIPAM mixtures for refining PNIPAM's mechanical properties. One such example is PNIPAM monomer crosslinked with *N,N*'-methylenebisacrylamide to form a hydrogel system composed of two PNIPAM networks. This system showed improved controlled release of bovine serum albumin [29]. Other studies which have fabricated PNIPAM and shown improved mechanical properties include polyethylene glycol (PEG)-conjugated-PNIPAM [30], poly(hydroxyethyl methacrylate)- conjugated-PNIPAM [31] and PNIPAM hydrogels containing poly(*N*-

isopropylacrilamide)-block-poly(methyl methacrylate) micelles [32]. However, when fabricating PNIPAM mixture systems, it is important to assess the shrinking-re-swelling cycle to determine the stability of the system [29, 33].

Another class of thermoresponsive polymers, which are widely used in ophthalmic preparations, are the poloxamers. These are non-ionic amphiphilic triblock copolymers known as polyoxyethylene-poly-oxypropylene-polyoxyethylene or PEO_A–PPO_B–PEO_A and are composed of a central hydrophobic unit of polyoxypropylene (PPO)(B) and two adjacent hydrophilic chains of polyoxyethylene (PEO)(A) [34]. Poloxamers are commercially available as Pluronics® and vary in their properties by their molecular weights and ethylene oxide-to-propylene oxide weight ratios [20]. For example, pluronic F127 has a molecular weight of 12 kDa and contains approximately 70% ethylene oxide, which contributes to its hydrophilicity and water solubility. It has a greater solubility in cold water relative to warm water, because of greater hydrogen-bond interactions and solvation at lower temperatures. The thermoreversible properties depend on the concentration of the poloxamer as well as the temperature, where solutions of concentrations higher than 20% w/w at temperatures exceeding 25°C have been reported to exhibit thermoreversible properties [35]. These thermoreversible properties coupled with gel formation at low temperatures restrict the ophthalmic application of pluronic F127 [36]. However, it has been shown to improve ocular drug permeation and hence improve the bioavailability of drugs [37]. Moreover, pluronic F127 displays optical clarity and has mucomimetic and protective properties, making it suitable for use as artificial tears [38]. To establish more applications for poloxamers, researchers tried to reduce their concentration and increase the phase transition temperature from ambient (25°C) to precorneal (33.8°C) temperature. One study achieved this by adding carbopol 974P (poly (acrylic acid) (PAA)), a pH-responsive gelling system generically known as carbomers (Section 2.2.2.), to the poloxamer 407 or 188 gelling systems. Gelation temperatures for these systems were in the range of 31.21-36.31°C. This combination provided additional benefits, such as enhanced drug solubility and mucoadhesive properties [39]. PAA has also been grafted onto pluronic F127 and showed prolonged precorneal residence time and improved ocular drug bioavailability [40]. Another study demonstrated reduced phase transition temperature and concentration of the poloxamer in combination with alginate [41].

Cellulose derivatives such as methylcellulose (MC),carboxymethylcellulose sodium (CMC) and hydroxypropyl methylcellulose (HPMC) are naturally occurring polymers with thermoresponsive properties. They are commercially available in various products for the treatment of dry eyes, including Murocel® (MC), Celluvisc® (CMC sodium) and Ultra Tears® (HPMC). However, unlike poloxamers, MC and HPMC aqueous solutions form gels at lower concentrations (1-10%) [42] and temperatures of 40-50°C and 75-90°C, respectively [43]. The phase transition temperature of cellulose derivatives decreases with a higher degree of cellulose ether substitution or with the addition of salts [44, 45]. The sol-gel transition temperature of MC is also influenced by the salt type and MC concentration [46, 47].

Combining cellulose derivatives with poloxamers generates gelling systems with enhanced properties to favour ophthalmic drug delivery. For example, pluronic F-127 in combination with HPMC, as a viscosity enhancing agent, prolonged the ocular residence time of the drug [48, 49]. Other polymers which are under investigation as single preparations or in combination with the aforementioned thermoresponsive polymers, to enhance ocular drug delivery, include xyloglucan [50, 51] and chitosan [52, 53].

2.2 pH-responsive polymers

pH-sensitive polymers contain ionisable groups allowing them to vary their solubility, undergoing sol-gel phase transition under different pH environments [54]. Cellulose acetate phthalate latex (CAP-latex) and carbomers (polyacrylic acid, PAA) are commonly studied pH-responsive polymers.

2.2.1 Mechanism of gel formation

Ophthalmic formulations contain pH-responsive polymers, also known as polyelectrolytes, which undergo sol-gel phase transition in response to environmental pH changes (i.e. pH of preparation *vs* pH of lacrimal fluid). The sol-gel phase transition results from changes in the ionisation state of the weakly acidic (carboxylic or phosphoric) or weakly basic (ammonium) groups present in the polyelectrolyte [55]. The pH at which these groups ionise depends on their *pKa* values (3 – 10) [56] and the molecular weights of the polymers [57]. Changes in the ionisation state of these groups lead to changes in conformation and solubility, as well as swelling of the system [58]. The gelling mechanisms and properties of some pH-responsive polymers are affected by the salt concentration, ionic strength and temperature. Horkay and Basser [59] showed that increasing the calcium concentration in a near-physiological solution containing PAA induced reversible volume transition. Other studies have demonstrated sol-gel transition in response to pH, ionic strength and temperature stimuli [60-62].

2.2.2 pH-responsive polymers used in ophthalmology

A common pH-responsive polymer which is widely used in ophthalmic preparations is PAA, and its derivatives (Table 1). PAA, also known as carbomer® or carbopol® [63], is an anionic high molecular weight synthetic polymer composed of acrylic acid cross-linked with either allyl ethers of sucrose or allyl ethers of pentaerythrityl [64]. Ophthalmic preparations containing PAA are formulated at low pH values and form gels in the higher pH environment of the lacrimal fluid. This transition is also dependent on the molecular weight of the PAA, where polymers of molecular weights greater than 16.5 kDa have shown sol-gel phase transition, whereas those with lower molecular weights have not [65]. However, it is important to note that formulations outside of the pH range of 4-10 cause ocular irritation and damage [66].

Table 1. Commercially available ophthalmic gel products. Adapted from [67].

Polymer(s)	Brand name	Company	Therapeutic agent
Carbomer	Pilopine HS®	Alcon Laboratories	Pilocarpine
	Zirgan®	Sirion Therapeutics	Ganciclovir
	Fucithalmic®	Concordia Int.	Fusidic acid
Carbomer 940	Pilogel®	Alcon Laboratories	Pilocarpine
Carbomer 980	Lumecare® Carbomer Gel	Medicom	Carbomer 980
	Viscotears®	Alcon	
	Xailin Gel®	Nicox	
	Clinitas Gel®	Altacor	
	GelTears®	Bausch & Lomb	
Carbomer 974P	Liquivisc®	Spectrum Thea	Carbomer 974P
Carbomer and polyvinyl alcohol	Nyogel®	Novartis	Timolol
Polycarbophil (carbomer crosslinked with divinyl glycol)	DuraSite®/ Azasite®	Inspire Pharmaceuticals	Azithromycin
	Azasite plus®		Azithromycin and dexamethasone
Gellan gum	Timoptic® XE	Merck	Timolol maleate
Xanthan gum	Timoptic® GFS	Alcon	Timolol maleate
Hypromellose	AktenTM	Akten	Lidocaine hydrochloride
Carbomer	Virgan®	Thea Pharmaceuticals Ltd	Ganciclovir
Polyethylene glycol	ReSure® Sealant	Ocular Therapeutix, Inc.	Trilysine acetate

PAA increases the viscosity of ophthalmic preparations but has also been reported to exhibit mucoadhesive properties [68] that further enhances ocular drug retention and delivery. These result from hydrogen bond formation between the carboxylic acid group of PAA and complementary functional groups of the mucus glycoprotein [69, 70] present in the conjunctiva and cornea [71] and is optimal at pH 4-6 [70]. To further improve the gelling properties and prolong drug release many studies have utilised a combination system, comprised of PAA with other viscosity enhancing agents; HPMC [72, 73] or MC [74]. PAA and MC have also been used to prepare pH-responsive interpolymer complexes or films with a tunable release for ophthalmic delivery of riboflavin [75].

Pseudolatex-based polymeric dispersions ocular formulations have been assessed for sustained ocular drug delivery [76]. One such example is cellulose acetate hydrogen phthalate (CAP)-latex which is an ion-exchange liquid at pH 4.4 and undergoes rapid transition to form a stable gel upon contact with the lacrimal fluid (pH 7.2) [54]. This has been used in conjunction with HPMC to construct a reservoir-type ocular insert for controlled delivery of acyclovir [77]. Further, CAP has low viscosity, good ocular tolerance and improved bioavailability, reducing the frequency of topical application and potentially improving patient compliance [54]. Taking these factors into consideration, CAP has significant potential for ocular drug delivery.

Various other pH-responsive polymers have also been investigated for ophthalmic applications, including chitosan, a pH-responsive polymer which also exhibits mucoadhesive [78] and permeation-enhancing [79, 80] properties, in combination with the thermoresponsive polymer (pluronic F-127) [81]. Others include; polyvinylacetal diethylaminoacetate [82] and poly(2-hydroxyethylmethacrylate-co-2-(diisopropylamino)ethyl methacrylate) [83].

2.3 Ion-activated polymers

2.3.1 Mechanism of gel formation

Anionic polysaccharides crosslinked with monovalent (Na^{+}) and/ or divalent (Mg^{2+} and Ca^{2+}) cations found in the lacrimal fluid may initiate sol-gel transition and increase polymer viscosity. The increase in polymer viscosity is directly proportional to the cation concentration [84, 85]. Therefore, an increase in tear production to dilute the viscous solutions would consequently result in an increase in cation concentration and hence, greater polymer viscosity, enhancing drug ocular retention time, reducing nasolacrimal drainage and improving drug bioavailability [86]. Examples of widely used ion-activated polysaccharides include gellan gum (Gelrite®/Kelcogel®) and alginic acid.

2.3.2 Ion-activated polymers used in ophthalmology

Gellan Gum is a deacetylated anionic extracellular polysaccharide obtained from *Pseudomonas elodea* and is composed of repeating units of α-L-rhamnose, β-D-glucuronic acid and two β-D-glucuronic acid residues [87]. At room temperature, in aqueous solution, this polymer forms double helices that are connected via weak van der Waals forces. Once in contact with the cationic electrolytes of the lacrimal fluid, these helices aggregate leading to cross-linking of the polymer and forming complexes with the cations and hydrogen bonds with the water [88-90]. This results in a conjunctival-scleral depot which allows for more controlled release of drugs [91].

Alginic acid is a biodegradable and biocompatible anionic polysaccharide composed of β-D-mannuronic acid and α-L-guluronic acid residues, linked by 1,4-glycosidic bonds, the sequence and proportions of which are determined by the algae source from which it is obtained [92, 93]. This polysaccharide increases the ocular drug residence time because of both its mucoadhesive nature and gel formation following the interaction of its guluronic acid residues with the Ca^{2+} ions of the lacrimal film [93-95]. The physicochemical characteristics of the formed gel are dependent on the concentration and viscosity of the alginate used, as well as the ratio of α-L-guluronic to β-D-mannuronic acid residues in its structure, increasing the content of the former would increase the extent of gelation [96].

3 Characterisation of ophthalmic preformed gels and *in situ* gelling systems

In addition to considering the effect of environmental stimuli in the development of *in situ* gelling systems, the polymers forming the gel must also have suitable characteristics for ocular application. These formulations should be biocompatible, safe, and biodegradable with no/minimal adverse effects. The formulation should have pseudoplastic flow with thixotropic characteristics, producing a low fluid viscosity under high shear rate conditions. This allows for ease of spreading across the ocular surface upon blinking and transforming to a highly viscous fluid under low shear rate conditions to prolong the ocular residence time [97]. Ideal properties of ophthalmic *in situ* gelling systems, together with their corresponding modes of investigation, are discussed below and summarised in Table 2.

Table 2 Characteristics and mode of investigation of topical ophthalmic formulations including preformed gels and *in situ* gelling (gel-forming) systems.

Characteristic	**Mode of investigation**	**Reference(s)**
Clarity and optical transmittance	Visual inspection and spectrophotometric analysis	[98]
Safety and good ocular tolerance	Observation for possible adverse effects Visual acuity	[99-101]
	Ocular tolerance (Draize, HETCAM, BCOP and histology tests)	[102, 103]
	Isotonicity test and cell-based cytotoxicity assays	
Suitable pH	Measurement of pH at 37°C	[13, 104]
Pseudoplastic and thixotropic flow Viscoelasticity	Cone and plate rotational or oscillation viscometer	[104-106]
Gelling capacity	Qualitative observation of gel formation in simulated tear fluid	[104, 107]
Mucoadhesive properties	Polymeric mucoadhesion tests	[107-110]

	Bioadhesive force of the gel	
Prolonged ocular residence time	Precorneal residence assessed using gamma scintigraphy	[103, 111, 112]
Ease and reproducibility of application	Gel consistency, firmness and cohesiveness assessed by texture analyser	[113]
Compatible excipients	Fourier transform infrared spectroscopy used to analyse potential drug-polymer interactions	[114]
Physical properties	Solid-state properties elucidated by X-ray powder diffraction and particle size analysed by dynamic light scattering	[115, 116]
Sustained drug release	*In vitro* dissolution studies	[100]
Enhanced transocular permeation	*Ex vivo* transcorneal and transscleral permeation studies	[7, 52]
Sterility	Direct inoculation method	[117]
Stability	Storage stability investigated	[118]

4 Hydrogel contact lenses

Hydrogels are the main constituent in contact lenses due to their high water content and favourable properties that render them highly compatible with human tissues [119, 120]. Hydrogel contact lenses are not only used for vision correction or cosmetic purposes, but they are also used as drug delivery devices for extended delivery of ophthalmic medications. Contact lenses prolong the resident time of their drug cargo, control and extend their release to several days or even months and enhance their bioavailability to more than 50% (conventional eye drops would achieve 5-10% ocular drug bioavailability) [121, 122]. These drug-eluting contact lenses can be particularly beneficial for chronic and elderly patients having trouble adhering to repeated dosage regimens characteristic of conventional eye drops where extensive treatment of serious conditions such as glaucoma, corneal ulcers or infections is required [123]. Moreover, drug delivery via hydrogel contact lenses can lead to reduced drug doses and thus side effects [124]. Several in-vivo studies, through animal models and small human trials, have proved the effectiveness of contact lenses as a drug delivery platform with improved clinical outcomes compared to conventional eye drops [125].

Contact lenses made from the hydroxyethyl methacrylate (HEMA) and its copolymers were used to extend the delivery of drugs to the eye for up to 1 week; drugs include timolol [121, 126], dexamethasone [127, 128], lidocaine [129, 130] and antifungal drugs [131, 132] . Other monomers including methacrylic acid (MAA), methyl methacrylate, poly (vinyl alcohol) (PVA) and N-vinyl-2-pyrolidone (NVP) can be incorporated into the hydrogel matrix to enhance the wettability of the lens

or to increase drug loading. [133]. For extended wear contact lenses, silicone hydrogels are used due to their high oxygen permeability compared to other polymeric hydrogels. The release of drugs from silicone hydrogel lenses can be extended from 20 days to 3 months by adjusting the ratio of the hydrophilic to the hydrophobic monomers incorporated [126].

Several methods have been employed to incorporate drugs in hydrogel contact lenses; the simplest method is soaking the lens in the drug solution [134, 135], where the drug is entrapped in the internal channels of the hydrogel. The soaking method is straightforward and cost-effective, it can be done with commercially available contact lenses or specially synthesised contact lenses from various polymer combinations [136]. However, the soaking technique is limited only to low molecular weight and hydrophilic drugs and suffer from low drug loading and fast release of the drug from the lens, mainly via diffusion [134, 137]. Vitamin E is an effective biocompatible diffusion barrier that can be used to prolong the release of the loaded drugs; it has proven to enhance drug stability and limit diffusion during storage [138]. Moreover, vitamin E acts as a UV barrier and can reduce water dehydration from the hydrogel lens [139].

To be able to incorporate lipophilic therapeutics, maximise drug loading and prolong the release from hydrogel lenses, several approaches have been employed including the addition of complexing agents such as β-cyclodextrin [140], surfactant aggregates [141] and nanoparticles [142, 143]. Molecular imprinting can offer another solution, through creating voids within the hydrogel macromolecular network with high affinity to the targeted drug, thus increasing the loading capacity and offering better control over the release of the incorporated drug [122]. The main drawback of molecular imprinting is the difficulty to incorporate more than one drug and the high degree of hydrogel crosslinking required, which might alter the physical properties or the oxygen permeability of the hydrogel lens [144]. Other approaches using multilayer contact lenses, sandwiching a drug-loaded PLGA polymer film between two layers of pHEMA hydrogel, were able to extend the release of the loaded drug for up to 1 month [131, 145].

5 Assessing the ocular tolerability of ophthalmic gels

Given the nature of the polymers, materials and excipients that are used in the preparation of ophthalmic dosage forms, it is paramount that any new eye formulation intended for topical ocular application, including ophthalmic gels, is investigated for potential toxicity/adverse effects on ocular tissues. Several tests and assays are adopted by various regularity bodies to determine the ocular toxicity and irritation potential of the used polymers; the most widely used tests are summarised below.

“Draize rabbit eye irritation test” is the oldest eye irritation test that was developed by Draize *et al.* in 1944 and is still widely used and approved by the Organisation for Economic Co-operation and Development (OECD) and the FDA to evaluate potential ocular irritation [146]. This test uses live rabbits (normally six rabbits) for the determination of the whole range of possible eye irritation effects from chemical substances. The irritation responses include; redness, swelling, oedema, cloudiness, discharges, haemorrhage and blindness [102, 147-149]. The test materials are classified based on a subjective scoring, from non-irritating to severely irritating, for their effect on the cornea, iris and conjunctiva [147].

Although the Draize test is still approved by many regulatory bodies, concerns have been expressed on ethical grounds [150]. The stress level and pain sensation caused by the test with the long duration of recovery and the need for large numbers of live rabbits have been criticised by animal welfare groups [150]. The main shortcomings of the Draize test is its subjectivity, poor reproducibility and the differences between the rabbit eye and the human eye [151]. Therefore, new tests were proposed based on recommendations of the National Research Council to refine the Draize irritation test and address the issues arising from it [102]. One of these tests is the low-volume eye irritation test (LVET), which was developed by Griffith *et al*.(1980) [152]. The test is a modified Draize test in which the number of test material is reduced while maintaining the same scoring system of the Draize test [152, 153]. Although LVET overcame some of the Draize test drawbacks, LVET is still criticised by animal welfare groups for the use of live animals. Moreover, if a negative irritation result is obtained using the lower amount, the standard procedure of the LVET requires increasing the amount of the test material and thus can reach the same level as the normal Draize test

Regulatory bodies have adopted a battery of *in vitro* and *ex vivo* irritation tests that can potentially replace the former controversial *in vivo* eye irritation tests. The developed test needed to be accurate and reproducible without the use of live animals. Over the past decade, numerous irritation assays were successfully developed and tested, amongst which are the isolated/ enucleated organ method such as the Bovine Corneal Opacity and Permeability (BCOP) test, the non-ocular organotypic models such as Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM), and the cell-based cytotoxic colorimetric methods such as Red Blood Cell (RBC) lysis and protein denaturation, Neutral Red Uptake (NRU) and Sulforhodamine B (SRB) assays. [154].

The BCOP test works by using ocular organotypic modules, which maintains (for a short time) the normal biochemical and physiological function of the enucleated eye [155]. Corneal opacity and permeability (Figure 2) are the main two responses measured by the BCOP test. Corneal opacity gives an indication of protein denaturation, damage to the epithelial and stromal layers of the cornea, swelling and vascularisation; all of which result from exposing the cornea to the test substance [155].

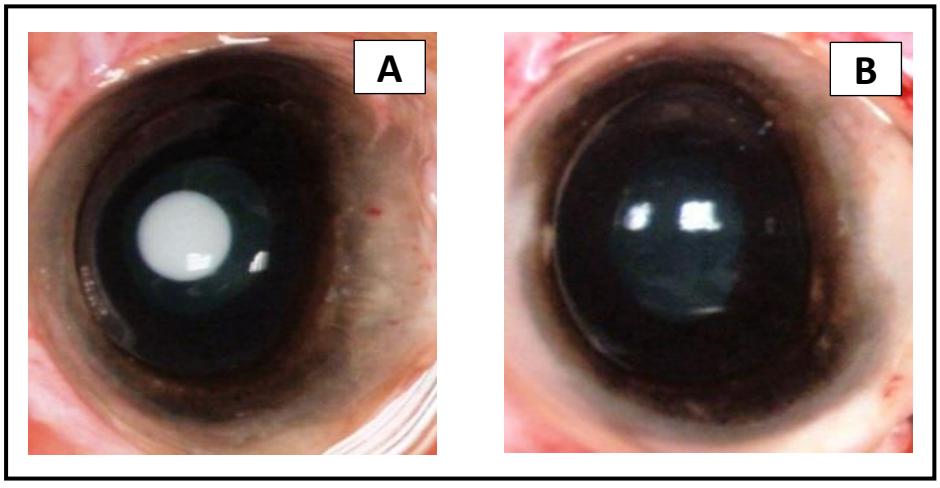

Figure 2 Change in bovine corneal opacity following the exposure to (A) Positive control which shows complete opacification of the cornea. (B) Negative control with no sign of corneal opacity change

Sodium fluorescein penetration gives an indication on the integrity of the corneal epithelial layer, damage by the test materials. Normally an intact and healthy cornea is completely impermeable to sodium fluorescein. The extent of the initial injury resulting from the test material correlates with the level of cell death. Therefore, the magnitude of the eye irritation is determined by the intensity of the initial injury [102]. As such, substances that damage the superficial corneal epithelial layer are classified as slightly irritant; substances that penetrate the corneal epithelium and stroma to reach and damage the endothelial layer of the cornea are classified as severe irritants; whilst substances that damage the epithelium and penetrate further to affect the stroma are classified as mild / moderate irritants.

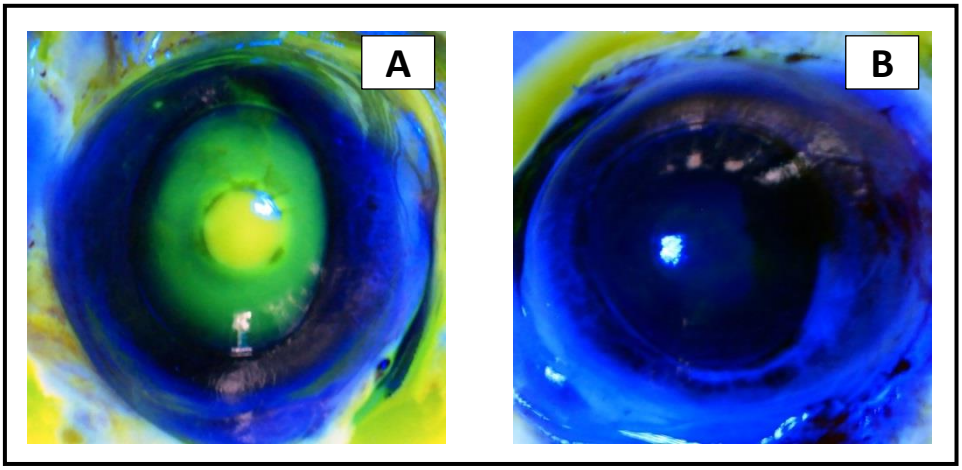

Figure 3 Fluorescein stained bovine's eye following the exposure to (A) Positive control which shows complete permeation of the fluorescein dye. (B) Negative control with no sign of fluorescein permeation

A histopathological examination of the corneal tissue after exposure to the investigated ophthalmic gel can provide a more comprehensive insight on the degree and depth of the corneal injury at cellular levels [156, 157]. The diffusion of the gel comprising materials (polymer, solvent, ions, and drug) through the main layers of the cornea (epithelium, stroma and endothelium) depends on the degree of the corneal injury. The corneal tissues are stained with Hematoxylin and Eosin (H&E stain), and signs of corneal damage are inspected, these include; epithelial injuries and stromal oedema. Such damages are perceived as sources of ocular irritation post topical treatment with the test material. Characteristic epithelial injuries that are documented and scored include cell loss, vacuolisation, nuclei coagulation (pyknosis) and Bowman's membrane cell separation (Figure 4) [150, 158]. Swelling, vacuolisation and pyknosis of keratocytes are the main characteristic features of stromal lesions [150, 158].

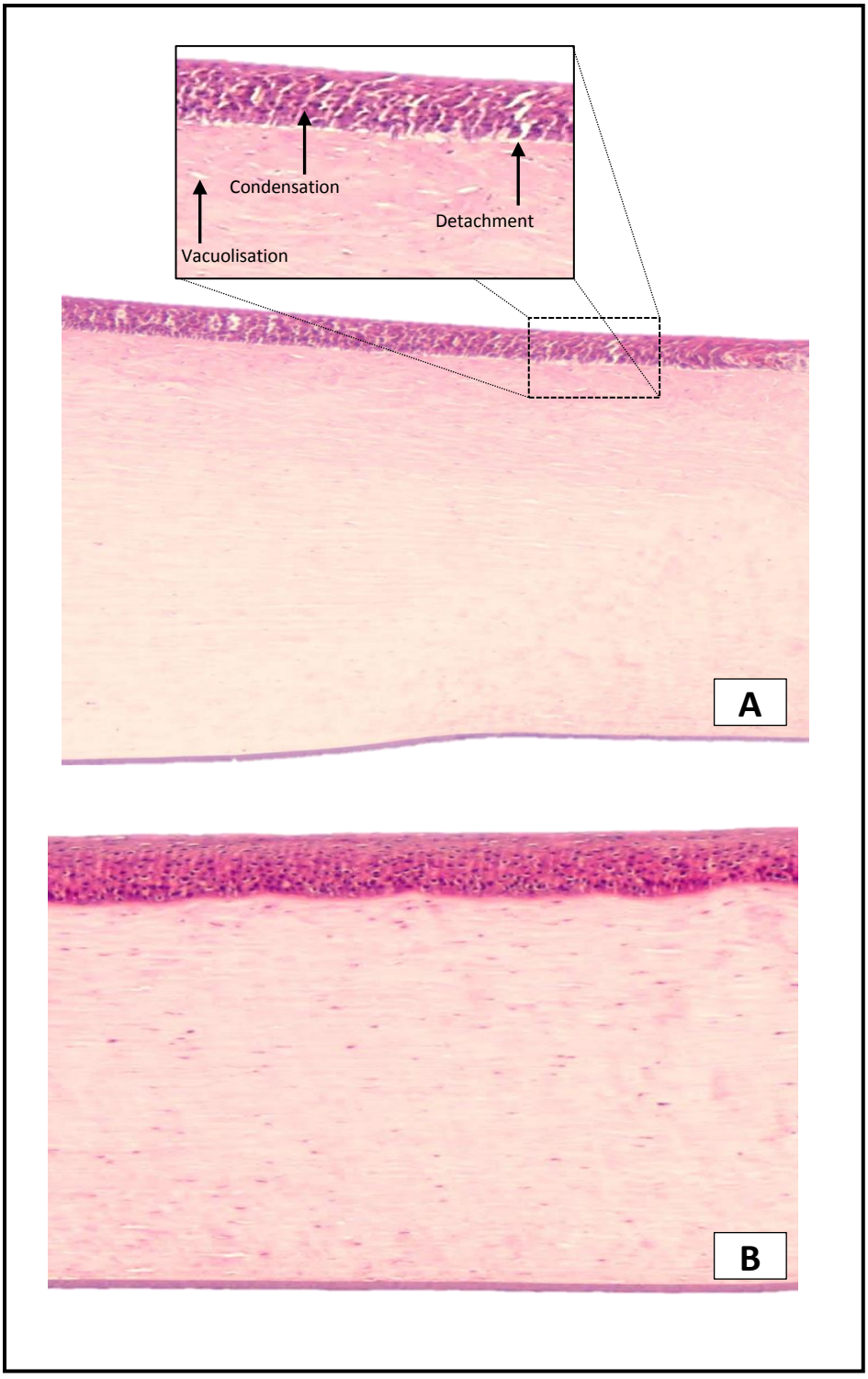

Figure 4 Histopathological documentation of H&E stained bovine cornea post exposure to an ocular insult. (A) Characteristic epithelial injuries that are observed and scored. (B) Negative control which shows normal histopathology of the cornea

The HET-CAM assay is another test that is widely used to assess the ocular irritation of ocular gels. The CAM is a vascularised respiratory membrane that surrounds the fertilised bird embryo; it has a network of arteries, veins and capillaries. The inflammatory responses of this blood vessel network to the gel formulation are similar to those of the conjfunctival tissue. As such, regulatory bodies recognise and accept the HET-CAM as a conjunctival model for the irritation test of ocular formulations including ophthalmic gels and *in situ* gelling eye drops [14, 15]. The test is fast, simple, inexpensive and reproducible; it has been successfully used to assess the irritation effect of several pharmaceutical systems [6, 14, 16, 17].

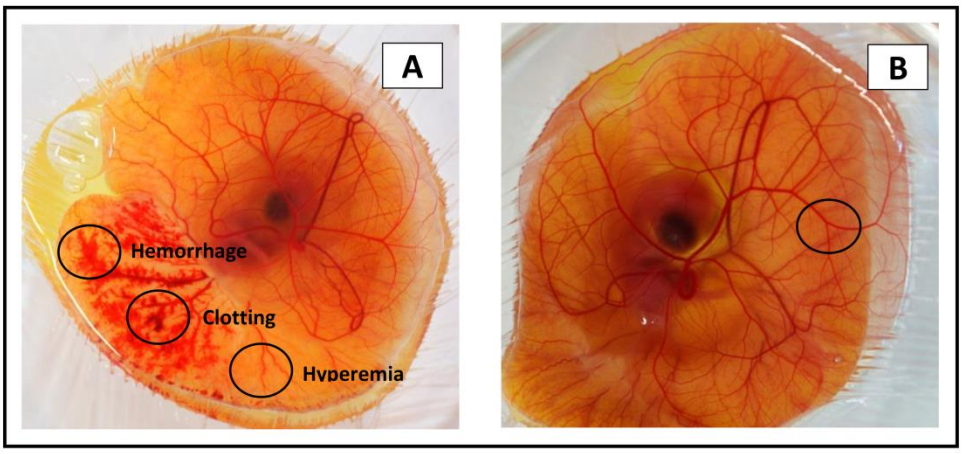

Figure 5 HET-CAM model showing (A) Inflammatory responses used to score conjunctival irritation. (B) Negative control with no sign of inflammation

RBC haemolysis assay has been used to test the irritation effect of substances. [159] This assay is fast, simple, and inexpensive with a defined endpoint that can be used as a screening method for potential irritation. The test is based on quantifying RBC haemolysis and protein denaturation of freshly isolated mammalian erythrocytes triggered by exposure to irritants that damage the cell membrane. [159-161] The cell membrane damage events in the RBC assay are well correlated to the initial inflammatory reaction and protein denaturation resulting in the corneal opacification due to the irritant effect of substances.[160] The oxyhaemoglobin (HbO2) released from the lysis of fresh red blood cells, due to the irritation effect, is measured using UV spectroscopy. The endpoint realtes to the concentration of the irritant materials that induces a 50% RBC lysis post exposure [160].

6 Newly developed ophthalmic gels and *in situ* gelling systems

Novel hydrogels are interesting biomaterials that are used for ophthalmic applications including drug delivery or as replacement corneal membranes. Their hydrophilic nature and resembling consistency to living tissues allow them to be biocompatible. Unfortunately, clinical applications of smart gels are still limited. It would be more useful and economical to develop novel hydrogels that meet the requirements of the desired applications rather than trying to find applications for newly developed hydrogels. Moreover, developing smart gels that mimic natural systems can advance and broaden their therapeutic uses [162].

Despite the development of a vast number of hydrogels that can be used for therapeutic purposes or medical applications, only a handful have been approved by the Food and Drug Administration (FDA) [162]. The FDA has set recommendations for assessing the safety of medical devices that can be relevant to hydrogel drug delivery systems including cytotoxicity, sensitization, hemocompatibility, pyrogenicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity tests [163].

A recently developed gel for drug delivery applications was made from pure proteins. The hydrogel was produced by the simple interaction between two recombinant proteins, ULD-TIP1 and ULD-GGGWRESAI. The porous protein hydrogels were evaluated by rheology, scanning electron microscopy (SEM) and cytotoxicity tests. Their degradation profile extended to 144 hours which is promising to extend the release of ophthalmic drugs [164].

6.1 Stimuli-responsive gels

Ophthalmic drug delivery via smart hydrogels, encompasses three types of stimuli; temperature, pH, and ions [165]. However, hydrogels can be stimulated by other environmental factors such as light [166], pressure [167, 168], thrombin [169, 170], antigen [171, 172], biomolecule [173, 174] or glucose [175, 176]. The unique properties of smart hydrogels open the door wide for novel applications including their potential use in ocular drug delivery. Currently, one of the critical limitations for the use of stimuli-responsive hydrogels in drug delivery is their delayed response time [177]. With further research and development of new hydrogels, this drawback can be overcomed.

A novel stimuli-responsive gel was designed by Yu et al. (2016) for ophthalmic drug delivery. The hydrogel was made by chemical cross-linking of the biocompatible and pH-sensitive carboxymethyl chitosan (CMC) with the temperature-sensitive poloxamer 407 (F127) using glutaraldehyde as a crosslinking agent to form a pH-induced thermosensitive hydrogel that can undergo a reversible sol-gel transition at a very low concentration. The gel maintained controlled release of a model drug and showed no cytotoxicity to human corneal epithelial cells [178].

Pressure-responsive hydrogels, such as poly (N-isopropylacrylamide), poly (N-n-propylacrylamide), poly (N,N-diethylacrylamide) and poly (N-isopropylacrylamide) tend to collapse at low pressure and expand at high pressure [177, 179-181]. In the future, pressure sensitive gels can be explored for controlling the drug release profile to adjust the intraocular pressure in glaucoma patients; this area is still open for new products. It is worth noting that pressure-sensitive hydrogels show sensitivity to change in temperature, this might be explained by the increase in the lower critical solution temperature (LCST) of the gels with pressure [180].

Most currently available smart gels have drug release profiles that are limited for few days. Hence, the need to develop improved hydrogels that may control drug release over a period of few months [162]. Osswald et al. (2015) have successfully developed an injectable, thermo-responsive gel that sustained the release of a model protein drug for 200 days. The drug was encapsulated within poly(lactic-*co*-glycolic acid) (PLGA) microspheres that were in turn suspended in the poly(N-iso-propylacrylamide) (PNIPA Am)-based hydrogel. The phase transition temperature of the hydrogel was maintained between 32 and 37 °C through adjusting the concentration of polyethylene glycol diacrylate (PEG-DA) in the cross-linking reaction [182].

6.2 Nanoparticle-hydrogel composites

The incorporation of ophthalmic drugs in nanocarriers has gained interest due to its potential to prolong ocular residence and sustain drug release. Furthermore, nanocarriers do not alter the transparency or the desirable ophthalmic characteristics like the case with suspensions or ointments [183]. Composites of polymeric nanoparticles in hydrogel matrices are appealing for controlled and extended delivery of hydrophobic drugs. The release profile of dexamethasone acetate was successfully prolonged from 3 to 30 days after loading into poly(ethylene glycol)- poly(ε-caprolactone) (PEG−PCL) micelles prior to incorporation in the hydrogel [184].

Novel biodegradable pentablock (PB) copolymer-based nanoparticles have proven to be effective in controlling and sustaining the release of loaded therapeutics to more than 100 days to treat posterior segment diseases. Polyglycolic acid-polycaprolactone-polyethylene glycol-polycaprolactonepolyglycolic acid (PGA-PCL-PEG-PCL-PGA) and polylactic acid-polycaprolactone-polyethyleneglycol-polycaprolactone-polylactic acid (PLA-PCL-PEG-PCL-PLA) are examples of PB copolymers that are non-toxic, non-immunogenic and biocompatible. The drug loading and release profiles of the loaded therapeutics can be controlled through altering the drug/polymer ratio and molecular weight, crystallinity and the block arrangement of the copolymers [185, 186]. To achieve constant zero-order release of their protein/peptides cargo, the PB nanoparticles were incorporated in biodegradable and injectable *in situ* thermosensitive hydrogels. The thermosensitive hydrogel offered high drug loading with minimum intravitreal injection volume, allowing for longer duration of action thus reducing complications and improving patient compliance.

6.3 Nanogels

Nanogels are hydrogels composed of a network of hydrophilic polymers and drug in the nanoscale range [187]. They can host the drug alone or when incorporated into liposomes, solid lipid nanoparticles, dendrimers or polymeric nanoparticles. Nanogels can hold large amounts of therapeutics by simple incorporation methods without altering the biological activity of their load [188], which can be useful for the delivery of peptides and antibodies. The hydrolytic degradation of the polymer network results in a sustained release of the entrapped drug [183]. The drug release from the nanogels can be controlled by adjusting the amount or type of polymers, charge on the drug or the nanogel, or via multiple environmental stimuli such as pH, temperature and ions [187, 189]. The combined effects of multiple stimuli on the nanogel network structure offer improved control over the release of the entrapped therapeutic agent. This can allow for various drug release profiles from the same hydrogel [183]. Furthermore, nanogels have enhanced permeability and retention effect compared to macrogels, which makes them preferred for delivery of nucleic-acid based drugs, to cells [190].

6.4 3D printed hydrogels

6.4.1 Tissue and organ generation

3D printing is a relatively new technology that allows the creation of physical three-dimensional objects with complex geometries from a computer-aided design (CAD) [191, 192]. The computer software splits the design into a series of thin horizontal cross-sections (slices) and sends them to the machine to print the designed structure layer-by-layer [193]. There is no need for fabrication moulds, which gives a high degree of freedom and flexibility in design such that structures that cannot be fabricated using conventional subtractive techniques can be made using 3D printing [194]. On the other hand, 3D bioprinting allows printing biomaterials to tissue analogue structures without any change in their mechanical or biological properties [195, 196]. 3D bioprinting is gaining increased interest in the medical field because it can fabricate tissues [197, 198], scaffolds [199], skin [200], cartilage [201, 202] and potentially, organs such as kidney and liver from human cells [203-206]. Those 3D printed tissues and organs are currently used in drug research and development [207], in medical schools [208] and for surgical applications. Co-culturing human retinal endothelial cells (HRECs) and human retinal astrocytes (HRAs) on 3D hydrogel scaffolds provided a more accurate representation for the blood-retinal barrier compared to previous 2D cultures [209].

3D bioprinting allows for the building of tissues and organs from the patient's own cells or induced Pluripotent Stem Cells (iPSCs) which eliminate immune responses. It can be utilised as the as an optionfor organ replacement to compensate the shortage and difficulties associated with donor transplantations [196, 210, 211]. The most common types of bioprinting techniques are ink-jet printing, extrusion-based printing and laser-assisted printing. In case of the ink-jet printing, the cartridge is filled with the biomaterial, similar to the conventional way of printing, and the bioink droplets are forced out from the nozzle by air pressure pulses to be deposited on specific locations on a substrate. Ink-jet printing was used to pattern stem cells on polymer substrates and to 3D print single or multiple cell types in a precise arrangement pattern [211-213]. With extrusion-based technique, the bioink is extruded through sterile syringes to create 3D biostructures [195]. Large structures with high cell densities can be formed using extrusion-based 3D printing technique. However, the resolution of the formed structures (200 µm) are less than that from ink-jet printing (20-100µm) and the high shear stress created when extruding the bioink through the tiny nozzle can affect cell viability [211]. Extrusion-based 3D printing technique was used to construct extracellular matrix of adipose, cartridge and heart tissues using human mesenchymal stem cells and embryonic stem cells [214, 215]. With laser-assisted bioprinting, the droplet of bioink is indirectly induced towards the substrate, with no mechanical stress, thus producing 3D printed constructs with high viability and resolution (5µm). Nevertheless, this technique is more expensive and time-consuming compared to the other techniques. 3D printed human osteoprogenitor cells and mesenchymal stem cells have been successfully printed using laser-assisted bioprinting technique [211, 216, 217].

3D bioprinting encompasses additional complexities compared with non-biological printing such as the choice of materials and the technical challenges related to the sensitivity of the materials and living cells used as bioinks [205]. Hydrogels are often employed as bioinks since they are printable, biocompatible and able to maintain cell viability and activity due to their ability to store huge amounts of water (up to 99% w/w), which mimics features of cellular environment [218, 219]. Bioinks can be made from polymers of natural origin such as alginate [220], gelatine [198], collagen

[221], fibrin [222], hyaluronic acid [223], chitosan [224] or agarose [225]; or it can be made from synthetic polymers including PEG [226] and Pluronic [227, 228]. The bioink employed in extrusion-based bioprinting has to be stimuli-responsive and self-supporting during layer-by-layer fabrication [229]. The shear thinning property of hydrogels helps in the extrusion of the bioink, where the hydrogels behave as liquid under high shear stresses during extrusion, but can easily turn into semi-solid gels, through thermal processes or post-print crosslinking [196]. It is important to evaluate the molecular weight, viscosity, gelation kinetics and stiffness of hydrogels used as a bioink for 3D bioprinting, [230]. Different types of hydrogels can be combined with various ratios to optimize the printing efficiency and resolution. Cross-linking of hydrogels is a critical step in the bioprinting process. This is why adding hydrogel precursors, which are reagents with chemically reactive, photo-polymerisable groups, to the bioink is important to help gelation during printing with the aid of UV/visible light with or without the effect of temperature [194]. Examples of such hydrogel precursors include poly(ethylene glycol) methacrylate, poly(ethylene glycol) diacrylate, poly(ethylene glycol) acrylate and gelatine methacrylate (GelMA) [225, 231].

In ophthalmology, the application of 3D bioprinting is still at its infancy and reported additive manufacturing for ophthalmic applications are very limited [211, 232]. However, this promising technology can be utilised to make corneas [196], ocular lenses [233] or other parts of the eye from the patient's own cells. It is expected that scientists can print on demand intraocular lenses, glaucoma valves and ocular implants using 3D printing and hydrogels as bioinks [192]. Human corneal epithelial cells (HCECs) were successfully printed into cell-laden constructs of collagen, gelatine and alginate to generate a bioengineered corneal epithelium using extrusion-based 3D bioprinting technique. This collagen/gelatine/alginate material system proved to be a successful bioink with high bioprinting resolution, good mechanical properties, and high cell viability [196]. Each of the aforementioned 3D printing techniques could lend itself to a specific ocular tissue construction application. For instance, layered corneal structures can be obtained by extrusion-based 3D bioprinting, with its ability to deposit multiple cell types with high cell density using multiple print heads. While fabrication of the limbal region would require the high resolution and speed of the ink-jet printing [211]. It is expected that the next generation 3D printed hydrogels will be adjusted to promote cell proliferation, differentiation and help induce extracellular matrix generation through release of cell-regulating factors [183].

6.4.2 3D printed drug delivery devices comprising ophthalmic gels

A drug delivery device (DDD) is a prefabricated system intended to release the drug to the desired organ with maximum safety, effectiveness, and reliability [234]. Delivery of therapeutics to the back of the eye using conventional eye drops is difficult due to dilution and clearance by the lacrimal fluid, nasolacrimal drainage, the need to diffuse through the vitreous and overcome the various blood-ocular barrier. These factors lead to poor ocular bioavailability of drugs and thus treatment failure [235, 236]. Intravitreal injections are used to deliver drugs to the posterior eye segment for treatment of diseases like age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa to reduce the risk of blindness [237]. However, intravitreal injections suffer from some complications such as increased intraocular pressure, intraocular haemorrhage and retinal detachment, together with poor patient incompliance [238]. Implantable ocular devices are only second to intravitreal injection when it comes to posterior segment drug delivery. They can maintain relatively high drug concentration at the target site with less side effects, yet have their own limitations [236].

One type of ocular devices is the implantable pump systems, where drugs are incorporated into an ophthalmic gel. The intake of biological fluids leads to swelling of the hydrogel and subsequent release of the drug in a controlled, continuous and sustained mode at the target sight [237]. However, the confined nature and relatively small size of the human eye adds a spatial constrain to the use of ocular devices, which could be resolved through the development of Micro-Electro-Mechanical System (MEMS) devices with drug-infused hydrogel systems [238, 239]. These MEMS devices have the advantage of controlled drug delivery rates despite their small size due to absence of the mechanical pump that is present in conventional pump systems [240]. 3D printing technology allows the construction of small, yet complex-structure drug delivery devices through layer-by-layer fabrication of structures with controlled porosity. These 3D printed DDDs are capable of holding therapeutics, including peptide nucleotides [241], and control their diffusion rate into the biological environment to achieve tailored drug release after implantation [242, 243]. Microbots are another example of these microscale DDDs that are used for the treatment of the anterior or posterior segment diseases via wireless manipulation and positioning of this tiny magnetic device. Fabrication of those complex shaped microbots is a challenge that can be further advanced through 3D printing techniques [244].

3D printing can be used to fabricate the drug delivery device itself or the moulds used for casting of the DDD using soft lithography techniques [191, 245]. Lee et al. (2012) developed an implantable ocular DDD consisting of micro/nanochannels embedded between polydimethylsiloxane (PDMS) top and bottom covers with a drug reservoir. The drug was entrapped in a hydrogel that is enclosed in the PDMS drug reservoir, which was fabricated using the replica-moulding technique from a master mould [237]. The master mould for the top and bottom layers of the reservoir were fabricated using a 3D printing stereolithographic technique, where a UV laser beam is used to solidify a photosensitive monomer liquid resin layer-by-layer to build a 3D structure [246].

6.5 New ocular applications for hydrogels

6.5.1 Production of artificial corneas

Human donor corneal transplants used to be the main treatment for severe corneal damage. However, corneal transplants have numerous limitations such as high cost and difficulty in storage. Moreover, patients might suffer from transplant rejection. Nowadays, new materials are developed as artificial corneas; hydrogels are biocompatible candidates that are used in ocular tissue engineering to replace defected corneas [247-249]. Poly (2-hydroxyethyl methacrylate) (PHEMA) is considered to be a potential substitute for the human cornea [248, 249]. Moreover, researchers have developed synthetic hydrogel corneal skirts with pores mimicking the structure under the limbal eye region to enhance cell binding, migration, adhesion, and elongation of human corneal fibroblasts, thus preventing keratoprosthesis extrusion [249-251].

6.5.2 Corneal wound healing

Ocular surface disorders that occur due to limbal stem cell deficiency (LSCD) can lead to impaired vision and blindness [252]. Currently, the main treatment of LSCD is amniotic membrane (AM) transplantation because of the anti-angiogenic and anti-inflammatory effects of the AM components [253-255]. However, the use of AM is often associated with limitations such as the need for extensive serological screening to ensure histological compatibility and to minimise the risk of possible disease transmission, impaired transparency, variable quality and poor mechanical strength [256, 257]. The biocompatible, viscoelastic properties of hydrogels render them a potential

candidate to replace AM. Moreover, their structure can be modified to match body tissues [252]. Various polymers have been utilised in tissue engineering of ocular surface through cross-linking to corneal stroma, such as poly(ethylene oxide) [258], alginates [259, 260] , gelatine [261], collagen [262-264], chitosan [262, 265, 266], fibrin [267, 268], keratin , cellulose and polymethacrylate [257, 269]. Thermoreversible PLGA-PEG-PLGA triblock copolymer hydrogels exhibit an interconnecting network structure at body temperature. This network acts as a substrate promoting epithelial cell migration during wound healing and thus can be used to substitute AM transplant in corneal wound healing [270].

7 Conclusion

Recent estimates of the global cost of sight loss suggest an annual figure of over US$3 trillion. The main disorders leading to sight loss are cataract, glaucoma, age-related macular degeneration (AMD) and diabetic retinopathy. Pharmaceutical formulation and drug delivery research have introduced promising eye treatments into the market; nevertheless, there remain unmet clinical needs and limitations associated with the performance of conventional ocular dosage forms. Compromised adherence and/or persistence with conventional eye drops that are applied topically to the surface of the eye is primarily related to the need to be administered once, twice (or even up to four times) daily, often as a combination of multiple drugs, to achieve their intended therapeutic purpose. Novel ophthalmic gels including nanogels, 3D printed hydrogels; medical devices tailored to deliver ophthalmic gels are promising to mitigate comorbidities associated with the aforementioned eye conditions. Production of artificial corneas and corneal wound healing are new and exciting areas of application of ophthalmic gels.

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