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Teaser Hydrogels providing sustained delivery of biologics to the back of the eye can make a significant impact in the treatment of many ocular diseases. This article reviews the properties, administration approaches, and development challenges for an ocular hydrogel delivery system.

Hydrogels for sustained delivery of biologics to the back of the eye

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Hydrogels are water-laden polymer networks that have been used for myriad biological applications. By controlling the chemistry through which a hydrogel is constructed, a wide range of chemical and physical properties can be accessed, making them an attractive class of biomaterials. In this review, we cover the application of hydrogels for sustained delivery of biologics to the back of the eye. In adapting hydrogels to this purpose, success is dependent on careful consideration of material properties, route of administration, means of injection, and control of drug efflux, all of which are addressed. We also provide a perspective on clinical and chemistry, manufacturing and controls (CMC) considerations that are integral to the development of an ocular hydrogel delivery system.

Introduction

Ocular drug delivery of biologics to the posterior segment of the eye is an important and rapidly developing field due to the growing need for treatments for ocular diseases such as age-related macular degeneration (AMD), retinal vascular diseases, posterior uveitis, and glaucomatous optic neuropathies [1]. Durable and effective drug delivery to the back of the eye remains a significant challenge. Delivery of biologics to posterior ocular targets is made especially challenging due to anatomical and physiological barriers, resulting in poor bioavailability at posterior tissues via anterior or systemic delivery routes [2]. The relatively small volume of the vitreous humor also dictates that delivery of drugs into the space are limited in volume to less than or equal to 150 μL [3], and that drugs clear relatively rapidly from this space into systemic circulation [4]. Innovative drug delivery approaches from nanoparticles, liposomes, micelles, hydrogels, dendrimers, microparticles, nanotubes, and implants have been evaluated. Few have made it to late stage clinical trials, with some recent exceptions such as the nondegradable implant reservoir, Port Delivery System [2]. Finding biodegradable drug delivery solutions that provide durable exposure to posterior tissues in a tractable delivery format remains a major unmet need.

Biologic drugs, whether isolated from natural sources or manufactured by recombinant DNA technology (i.e., biotechnology), are sensitive to environmental conditions including pH,

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temperature, salt concentration, shear forces, and exposure to water/solid/air interfaces, further complicating the afore-mentioned design constraints for posterior ocular drug delivery [5]. Because of this, hydrogels can be seen as a good choice for encapsulation and release of biologics [5]. Hydrogel properties can be tuned over a relatively wide range and can be designed to provide a close mimic for biological matrices, exhibiting many similar properties including high hydrophilicity, high hydration, and closely matched mechanical properties [6]. These characteristics may also serve to preserve the physicochemical state of biologic drugs over extended times, especially when compared to more rigid, hydrophobic polymer matrices that can be used for drug encapsulation and release (e.g. well-reported degradation of peptides and proteins in solid matrices based on biodegradable poly(lactide-co-glycolide) (PLGA)) [7].

Various hydrogels have been used in a large number of drug delivery systems, in particular for oral drug delivery [8,9]. Hundreds of oral sustained release drug delivery systems exist for clinical applications. On the other hand, the use of hydrogels for non-oral routes of delivery have not been as extensive, and in fact, have been very limited. In the realm of ocular drug delivery, despite a long record of literature reports of hydrogels designed for this purpose, there remains no marketed hydrogel-based ocular drug delivery products. This absence is due primarily to the challenge of meeting a strict set of performance requirements including safety, tolerability, manufacturability, degradability and ease of administration, while maintaining activity of the encapsulated drug and achieving a clinically-relevant drug release profile.

Despite the absence of marketed products, the promise of hydrogels as ocular drug delivery systems remains. Hydrogels are generally considered to be highly biocompatible due to the excess of water present within the system, which is thought to mimic biological matrices [10]. Hydrogels can also be designed to provide short- or long-term release of drugs including peptides and proteins [11–16], whether by physical or chemical control. Modern-day hydrogels, including ''smart'' or environment-responsive hydrogels [17], have properties that were not available decades ago, and hydrogels with improved properties have made it possible to consider ocular drug delivery for durations of weeks or months while overcoming challenges of limited modes of administration.

In this review, we describe hydrogel characteristics and physiochemical properties for delivery of biologics to the back of the eye. The physiological barriers of the eye and the applications of hydrogel via different routes of administration are discussed. Moreover, different classes of hydrogels, from stimulus responsive to injectable *in situ* forming gels, are reviewed. In addition, clinical and manufacturing challenges anticipated during the development of a hydrogel ocular delivery system are discussed.

Hydrogel characteristics

A hydrogel is a three-dimensional network of hydrophilic polymers that absorbs water and swells but exists as a solid material. Hydrophilic polymers interact favorably with water molecules through backbone or pendant hydrophilic chemical structures, generally resulting in a high water solubility. In a hydrogel, hydrophilic polymer chains are made to form a network which retains the favorable water interactions but resists dissolution through chemical or physical crosslinks. A crosslinked three-dimensional polymer network swells in water for the same reason that an analogous linear polymer mixes with water spontaneously to form an ordinary polymer solution; the swollen hydrogel is in fact an elastic solution rather than a viscous one [18]. Hydrophilic polymers that are commonly used to form hydrogels include synthetic polymers, such as poly(hydroxyethyl methacrylate) (PHEMA), poly(ethylene oxide) (PEO, also called poly(ethylene glycol) (PEG)), polyvinylpyrrolidone (PVP), poly(acrylic acid) (PAA), polyacrylamide (PAM), and poly(vinyl alcohol) (PVA), and natural polymers include agar, gelatin, fibrin, hyaluronic acid (HA), alginic acid, carboxymethylcellulose (CMC), and hydroxypropyl methylcellulose (HPMC).

A hydrogel network can be formed by either covalent or non-covalent bonds among polymer chains to form chemical or physical hydrogels, respectively [19]. Examples of non-covalent bonds that can be engaged include hydrogen bonding, hydrophobic interactions, and physical entanglements. A hydrogel can be a homogeneous network of the same polymer, or it can a mixture of two or more different polymers. If each of the two different types of polymers makes its own network through covalent bonds, the overall structure is called an interpenetrating network. If one of the two networks is not covalently crosslinked, the structure is known as a semi-interpenetrating network.

History of hydrogels

According to SciFinder®, the first articles that used the term ''hydrogel'' were published in the mid-1890s to describe gelatinous ferric hydroxide [20] of silica gel [21]. Hydrogels made of hydrophilic polymers were first described in 1900 using the alcohol-gelatin-water ternary system [22]. The concept of hydrogels that we deal with nowadays, however, began in 1960 when Wichterlie and Lim published their paper on hydrophilic gels for biological use [23]. Hydrogels in their early stage of development possessed only one function: absorbing water and swelling. Recent advances in polymer chemistry have produced numerous new hydrogels that possess additional functions, such as the ability to respond to changes in environmental factors. These ''smart'' hydrogels swell, change shape, undergo sol-gel phase transition, or degrade in response to various environmental stimuli [24,25]. These properties have been used to develop stimulus-responsive drug delivery systems, as will be described in a later section.

Physicochemical properties of hydrogels

Physical properties of hydrogels

Water absorption and subsequent swelling in the presence of abundant water is the most fundamental property of hydrogels. The extent of water absorption, and thus, swelling of a hydrogel, varies significantly between hydrogel chemistries. The extent of swelling is usually measured by the swelling ratio (Q) which is defined by the weight (or volume) of a swollen hydrogel (Q_s) divided by the weight (or volume) of a dried gel (Q_d). The Q value of hydrogels can vary from slightly higher than 1 to more than 100. There is no predefined extent of swelling that makes a material a hydrogel. The extent of swelling depends on many factors, including the nature of hydrophilic groups and degree of crosslinking. As the crosslinking density increases, the swelling ratio decreases. Thus, whether a material can be considered a

hydrogel is not based on the extent of swelling or the amount of absorbed water. Rather, it is based on the nature of polymer chains. If polymer molecules in a hydrogel can dissolve in water in the absence of crosslinking, the crosslinked network can be called a hydrogel. In the presence of a limited amount of water not sufficient to dissolve all the polymers, even non-crosslinked polymers in a partially swollen state can be called hydrogels.

Because a hydrogel is a crosslinked network, it behaves like a solid material, and thus, it does not flow. An exception to this can occur if the crosslinking density of a hydrogel is so low that the solid characteristics cannot be maintained. At very low crosslinking density, polymer chains behave more like a viscous solution than a gel. Even in the absence of any crosslinking, hydrophilic polymers can form a transient hydrogel. For example, a matrix of compressed hydrophilic polymers, such as poly(acrylic acid) or hydroxypropyl methylcellulose (HPMC), can absorb a small quantity of water to form a hydrogel network. However, this network is only transient; as more water is absorbed, polymer chains disentangle and the network dissociates. This transient crosslinking behavior can be either an advantage or a disadvantage depending on the intended application.

Sensitivity to external stimuli

Hydrogels can be designed to undergo a pre-defined change in response to external stimuli [17]. As illustrated in Fig. 1, hydrogels can swell or deswell, degrade, change shape and undergo phase transitions in response to environmental or external stimuli. Hydrogel swelling and deswelling can be dictated by changes in hydrophobic interactions as a function of temperature. Hydrogel degradation can occur through cleavage of bonds within the hydrogel network either through enzymatic or chemical means. Hydrogels can change their shape in response to physiological conditions, e.g., if two hydrogels with different swelling properties are layered. Finally, hydrogels can transition between solution phase and gel phase based on physical crosslinking among polymer chains. For example, gelatin can act as a thermosensitive hydrogel, since it dissolves at room temperature but forms a gel at lower temperature. The inverse case, referred to as inverse thermosensitive hydrogels, are soluble at lower temperatures and transition to a gel with increasing temperature. These polymers typically possess hydrophobic moieties, the hydrophobic character of which increases with temperature. At a certain temperature, these interactions will result in formation of a physical network through hydrophobic chain entanglements [26].

TABLE 1

Examples of external factors affecting hydrogel properties

Chemical factors	Physical factors	Biological factors
pH	Temperature	Antibodies
Ion type	Light	Enzymes
Ionic strength	Electrical field	Glucose
Organic solvent	Magnetic field	Ligands
	Ultrasound	

There are various external factors that can be used to alter the properties of smart hydrogels (Table 1). The external factors can be grouped into three categories: chemical, physical, and biological. Various environmentally sensitive polymers have been used for ocular drug delivery for enhanced ocular retention of the administered drug and sustained release [27]. Of the factors listed in Table 1, temperature may be the most relevant for ocular drug delivery. A polymer solution at room temperature can be administered to the eye to form a hydrogel at body temperature. Commonly used temperature-responsive polymers are tri-block copolymers of PEO and poly(propylene glycol) (PPG), which are commercially available under Poloxamers (manufactured by ICI) and Pluronics (manufactured by BASF), N-isopropylacrylamide copolymers, and copolymers of PEO and poly(lactic-co-glycolic acid) (PLGA) [28].

Routes of hydrogel administration

Ocular drug delivery is a particular challenge due to the unique physiological and anatomical barriers of the eye [2]. Various forms of ocular drug delivery systems have been developed for clinical use and pre-clinical investigation and can be divided based on the

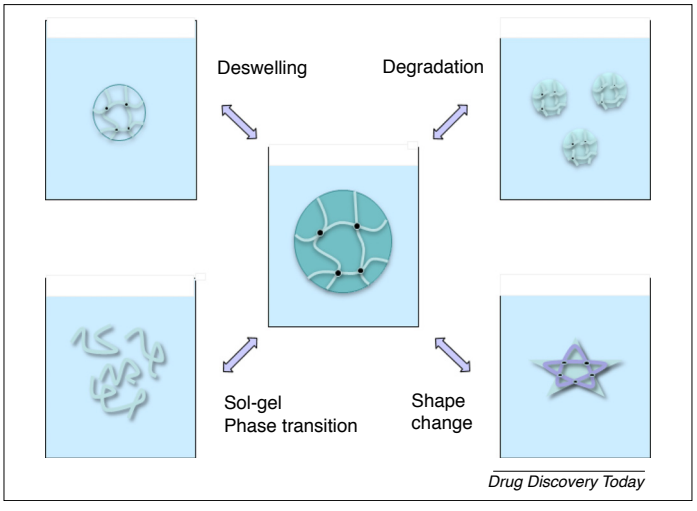

FIGURE 1

Hydrogels can be designed to respond to various environmental factors. A hydrogel can undergo deswelling, degradation, gel-to-sol phase transition, and shape change. These processes are generally repeatable with the exception of degradation.

routes of administration into systemic delivery, extraocular delivery, and intraocular delivery [29]. Extraocular delivery occurs either by topical delivery or subconjunctival delivery. On the other hand, there are many methods for intraocular delivery, including intrastromal, intracameral, intrascleral, intravitreal, suprachoroidal, and subretinal delivery [29], as shown in Fig. 2. The relevant ocular barriers depend on the route of administration. The corneal epithelium is the primary tissue barrier restricting drug penetration after topical administration, the conjunctival epithelium and associated tight junctions restrict subconjunctival administration, and the internal limiting membrane (ILM) and retinal pigment epithelium (RPE) are the limiting barriers for intravitreal administration [30]. The efficacy of a specific drug delivery system will depend in part on the route of administration, the pharmacokinetic parameters (such as bioavailability and clearance) at the target site, and the potency and release rate of the drug [31]. Protein permeability through different ocular tissues have been reviewed [32] and this can greatly affect the uptake and clearance of the biotherapeutic. The use of hydrogels as drug delivery vehicles can enhance the bioavailability by increasing the contact time or serve as a sustained release depot for the drug. The uses of hydrogels through different routes of delivery are discussed below (Table 2).

Topical delivery from hydrogels

Topical delivery, e.g. via ophthalmic drops or contact lenses, is the most non-invasive route of delivery. However, due to limited penetration to posterior tissues, topical delivery is typically used for external, corneal, and anterior segment diseases, and only a few studies have suggested efficacy for posterior segment diseases [29]. One of these studies showed intraocular penetration of a single chain antibody with a molecular weight (MW) of 26 kDa after topical application in a rabbit model [33]. Intraocular penetration to the posterior and anterior segment of the eye was found to occur with a frequent dosing regimen with and without a penetration enhancer. Topical delivery of protein therapeutics to the posterior segment is limited to peptides or proteins with low MW [34]. It requires highly concentrated formulations because of the low bioavailability (<5%) arising from poor drug penetration and loss from tear lacrimation [2].

For topical delivery, hydrogels can be used to increase the contact time of the drug formulation with the cornea. Examples include hydrogels in the form of contact lenses or viscous solutions. Several studies have evaluated the delivery of proteins to the posterior segment of the eye through hydrogel contact lenses [35–38]. Rabbits fitted with drug loaded hydrogel contact lenses showed detectable drug levels in the posterior segment of the eye for both small molecule drugs and larger biological molecules such as ranibizumab [37]. A clinical study using the Boston Ocular Surface Prosthesis (BOSP) lens, a large diameter, rigid, gas permeable lens, to deliver bevacizumab showed improved visual acuity [35]. The BOSP contact lens improved bioavailability by prolonging the drug exposure time to the eye.

Intravitreal delivery from hydrogels

Intravitreal (IVT) injection is performed by direct injection of drug into the vitreous chamber of the eye. IVT has been the preferred route of administration for posterior targets because it can achieve high drug concentrations in the vitreous and potentially high bioavailability to posterior tissues such as the retina. The injection procedure is typically done in a clinical setting with a 27 or 30 G needle. The injection volume in humans is typically equal to or less than 100 μL to limit the transient elevation in intraocular pressure that results from an intravitreal injection [3].

IVT injection of biologics still requires repeated injections, which carry the risk of complications such as endophthalmitis, retinal detachment, iritis/uveitis, intraocular hemorrhage, ocular hypertension, cataract, and hypotony [39]. Novel long-term controlled release systems, such as drug-encapsulating hydrogels, may reduce the injection frequency and improve patient compliance.

IVT injection of a hydrogel for long-term delivery of proteins requires the hydrogel system to be injectable, biodegradable, and biocompatible. Most importantly, the hydrogel system must demonstrate sustained delivery of protein therapeutics for up to several months. The hydrogel delivery system can be an *in situ*-gelling

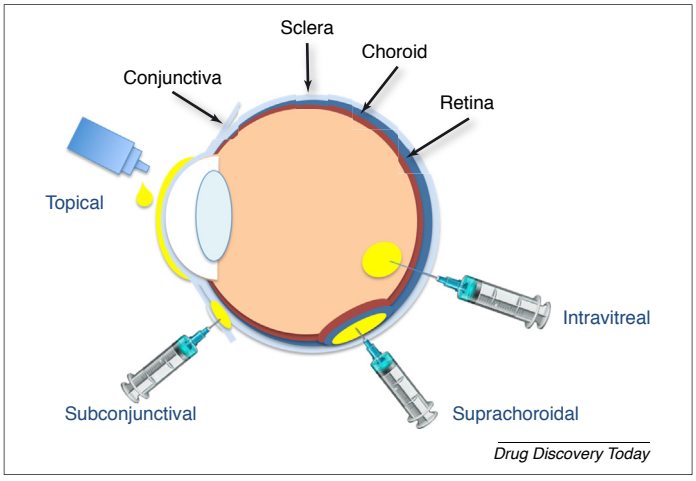

FIGURE 2

Hydrogels have been delivered via various routes of administration to access different sites of action. The route of administration is dictated both by the form of the hydrogel (e.g. monolithic depot vs. micro/nano-gel) and the therapeutic needs of the drug being delivered (e.g. site of action in retina vs. choroid).

TABLE 2

Examples of hydrogel delivery systems designed for sustained release of biologics

Hydrogel System	Drug	Release duration	Release Mechanism	Remarks	References
Alginate microspheres within a collagen hydrogel	BSA	11 days	Diffusion	Alginate microsphere encapsulated within a collagen hydrogel for sustained delivery of BSA	Liu, 2008 [84]
2-hydroxyethyl methacrylate and 2-aminoethyl methacrylate p(HEMA-co-AEMA)	BSA	12 days	Diffusion and swelling	Nanocomposite contact lens made of gelatin nanoparticle grafted onto p(HEMA-co-AEMA) to encapsulate hydrophilic protein drug	Zhang, 2013 [85]
Tetra-PEG with ß-eliminative linkers	Exenatide, peptide	a few hours to over a year	Self-cleaving linker	ß-eliminative linkers to tether drugs and crosslink PEG hydrogel for sustained release and controlled hydrogel degradation	Ashley, 2013 [42]
pNIPAAm-dextran	BSA	15 days	Diffusion through thermoresponsive hydrogel	Thermoresponsive and biodegradable hydrogel for aqueous encapsulation and release of hydrophilic drug	Huang, 2005 [86]
4-arm PEG	Bevacizumab	14 days	Diffusion and hydrogel degradation	*In situ* crosslinked PEG hydrogel from 4-arm PEG-Mal and 4-arm PEG-SH	Yu, 2014 [68]
Polycaprolactone dimethacrylate (PCM) and hydroxyethyl methacrylate (HEMA)	Bevacizumab	4 months	Diffusion and hydrogel degradation	Light activated, *in situ* forming PCM and HEMA based hydrogel for sustained suprachoroidal delivery of bevacizumab	Tyagi, 2013 [48]
Crosslinked alginate and chitosan	Lysozyme Bevacizumab,	18 days 3 days	Diffusion and erosion	Injectable *in situ* crosslinked polysaccharide hydrogel	Xu, 2013 [41]
pNIPAAm crosslinked with PEG-DA	BSA, IgG	21 days	Diffusion through thermoresponsive hydrogel	Crosslinked thermoresponsive hydrogel	Kang Derwent, 2008 [60]
PEG-heparin hydrogel	Heparin	49 days	Retro-Michael reaction	Hydrogel made with reversible maleimide-thiol linkage that is sensitive to reducing environments	Baldwin & Kiick 2013 [87]
Triblock copolymer (PEO)z-PCL-(PEO)z	Bevacizumab	20 days	Diffusion and hydrogel degradation	Thermal responsive biodegradable triblock copolymer with reversible sol-gel transition for drug encapsulation and sustained release	Wang, 2012 [61]
Hydrogel contact lenses	Ranibizumab	11 days	Diffusion	Hydrogel contact lenses for delivery of ranibizumab	Schultz, 2011 [37]
PEG-poly-(serinolhexamethylene urethane) (ESHU)	Bevacizumab	9 weeks	Diffusion	Thermal responsive ESHU gel for sustained release of bevacizumab	Rauck, 2014 [40]
PNIPAAm crosslinked with Acryl-PLLA-PEG-PLLA-Acryl	IgG, Bevacizumab, Ranibizumab	10 days	Diffusion through thermo-responsive hydrogel	Glutathione-modulated degradation of and release from pNIPAAm-based thermal responsive hydrogels	Drapala, 2014 [56]
Enzymatically responsive PEG hydrogel	Peptide	10 days	Enzymatic (MMP) cleavage	PEG hydrogel with enzymatically degradable sequence for delivery of peptide drug	Van Hove, 2014 [43]
Pentablock copolymer	IgG-Fab	80 days	Diffusion and hydrogel degradation	Pentablock copolymer (PCL-PLA-PEG-PLA-PCL) nanoparticle suspended in thermal responsive copolymer gel	Agrahari, 2016 [88]

TABLE 2 (*Continued*)

Hydrogel System	Drug	Release duration	Release Mechanism	Remarks	References
Crosslinked hyaluronic acid/ dextran	Bevacizumab	6 months	Diffusion and hydrogel degradation	*In situ* hydrogel formed by crosslinking between vinylsulfone functionalized hyaluronic acid (HA-VS) and thiolated dextran (Dex-SH)	Yu, 2015 [44]
PLGA microspheres suspended within PNIPAAm-PEG-DA hydrogel	Ranibizumab Aflibercept	200 days	Diffusion and hydrogel degradation	Injectable, thermal responsive microsphere-hydrogel combined system	Osswald, 2016, [89] 2017 [90]
PLGA microsphere in PEG-PLLA-DA/NIPAAm	Ranibizumab Aflibercept	180 days	Diffusion and hydrogel degradation	Physical and mechanical characterization of biodegradable microsphere-hydrogel system	Liu, 2018 [77], 2019 [78]

pNIPAAM: poly(*N*-isopropylacrylamide); PEG: polyethylene glycol; PLGA: poly(lactic-co-glycolic acid); PCL: polycaprolactone; DA: diacrylate; PLLA: poly(L-lactic acid); PEO: poly(2-ethyl-2-oxazoline).

formulation where upon injection the gel undergoes phase transformation by an external stimulus, such as temperature, pH, and ionic composition. Once injected into the vitreous, the *in situ* gelling system forms a depot where drug slowly diffuses/releases from the gel formulation. The hydrogel can also be formed *ex situ* into microparticles or in monolithic form and broken up for injectability. The size, shape, stiffness and surface chemistry of the hydrogel can be modified and engineered to increase biocompatibility. The injected hydrogel may cause clouding of the vitreous if freely floating and non-transparent. If the density of the hydrogel is slightly higher than that of the vitreous humor, it may settle at the bottom of the vitreous and not disrupt the visual path. However, physical contact with the retina could increase the risk of retinal damage or other adverse findings, which must be assessed through pre-clinical testing.

IVT injection of a novel hydrogel delivery system may hold great promise for delivery of proteins to the back of the eye. Many new hydrogel ocular delivery platforms have emerged in recent years. A recent paper [40] showed *in vivo* sustained release of bevacizumab from a thermal responsive gel made from poly(ethylene glycol)-poly(serinol hexamethylene urethane) block copolymer after IVT administration. The drug is physically encapsulated by the polymer matrix and is released when the polymer backbone degrades. Another study demonstrated an *in situ*-crosslinked polysaccharide for bevacizumab released by mixing oxidized alginate and glycol chitosan [41]. Injection of the mixture into the vitreous in the sol state resulted in gel formation and showed sustained release of bevacizumab for up to 3 days. This relatively short release duration may not be clinically viable, which demonstrates the typical challenge for delivering biologic by physical entrapment within the hydrogel matrix. Namely, can enough drug be loaded into the delivery system and the release from the matrix be slowed enough to enable long-term, i.e., multiple months, release of a clinically relevant quantity, or effective dose of an ophthalmic drug?

To prolong the release duration, some groups have explored chemical conjugation of the drug to the hydrogel. Cleavable linkers have been used to conjugate drugs to poly(ethylene glycol) (PEG) hydrogels for half-life extension, where the drug release and hydrogel degradation is tunable by varying the linker chemistry [42]. A different linker design that is enzymatically responsive showed release of therapeutic peptides from a PEG hydrogel upon exposure to matrix metalloproteinase (MMP) [43]. Yu *et al.* showed in-vivo 6-month release of bevacizumab from chemically crosslinked hyaluronic hydrogel after intravitreal injection in rabbit eye. The hydrogel showed good biocompatibility over the 6-month time frame with no increase in intraocular pressure or inflammation and retinal damage [44].

Suprachoroidal delivery from hydrogels

The suprachoroidal space is an artificially created, or ''potential'' space between the sclera and choroid, formed by injecting material into the site, thereby separating these layers. Direct suprachoroidal injection localizes the therapeutic agents at the site of action to provide higher drug exposure to the choroid and retina. Injection of a hydrogel sustained delivery system can limit the otherwise fast clearance of biologics [45] through the choroid blood flow to maintain constant drug exposure [46], while systemic exposure to the administered drug is comparable with intravitreal injections [36].

Due to the sieving effect, intact particles ranging from 20 nm to 10 μm that are injected into the suprachoroidal space remain there for months [47]. This suggests that biodegradable hydrogel particles can be used for sustained delivery by this route of administration, such that intact particles will stay in the space and clear when degraded.

Studies with a light-activated *in situ* forming gel of poly(caprolactone dimethacrylate) (PCM) and hydroxyethyl methacrylate (HEMA) showed sustained suprachoroidal delivery of bevacizumab for up to 4 months in rat eyes [48]. However, the light-activated system has the limitation of free radical generation that could cause damage to the eye.

Subconjunctival delivery from hydrogels

Localized drug delivery to the posterior segment of the eye can also be achieved through subconjunctival injection of a hydrogel at the space between the conjunctiva and sclera. Subconjunctival injections have greater bioavailability than topical administration [36] and can be less invasive than intravitreal injection, and it has combined merits of both administrations. Studies have demonstrated sustained release of insulin with a subconjunctivally implanted hydrogel [49]. These hydrogels are biodegradable and

thermoresponsive, and showed sustained release for up to one week, with no inflammation or evidence of toxicity in the *in vivo* rat and *in vitro* retinal cell model.

To achieve efficacious delivery of a biologic into the retina from a subconjunctival injection requires knowledge of protein penetration across the intraocular tissue. Studies with C14 insulin injection showed the dominant penetration pathway through direct diffusion across the sclera into the posterior segment of the eye [50].

To enhance drug penetration into the eye, transscleral iontophoresis has been studied [51]. Iontophoresis uses small electric currents to enhance charged molecule penetration. Antibiotic-loaded hydrogels coupled with a short iontophoretic treatment increased drug penetration to the eye [52]. However, delivery of high MW compounds to the inner retina via transconjunctival iontophoresis was not as effective *in vivo* [32].

Classes of injectable hydrogels

The most effective way to maximize posterior protein bioavailability is via injection into the vitreous body, the clear gel in the vitreous chamber, or into other posterior spaces. As a result, significant interest has been focused on designing dynamic hydrogel chemistries that can undergo a change in physicochemical properties upon injection. While this is an extremely active area of research in the broader hydrogel field, those that possess the greatest promise for posterior ocular applications are stimulus-responsive hydrogels, shear-thinning hydrogels, *in situ* gelling systems and nano- or micro-gels.

Stimulus-responsive hydrogels are attractive for biomedical applications because they can respond to specific chemical, physical or biological cues [53]. Hydrogels that undergo a physicochemical change in response to pH, temperature or light take advantage of the nonlinear response of certain chemical properties to these stimuli. In this way, even relatively minor environmental changes can result in a significant change in hydrogel properties.

Understanding how to take advantage of this stimulus-responsiveness is paramount in designing an effective system. In the ocular realm, stimulus-responsiveness is generally used to achieve a sol-gel transition between the liquid pre-injection state of the system and the solid post-injection state. In this way, a solution that is readily injected into the vitreous can then respond to, e.g. the increase in temperature from room to body temperature and solidify into a cohesive gel.

Thermosensitive hydrogels

Temperature-sensitive polymers are characterized by a critical temperature at which their sol-gel behavior undergoes a drastic change [28]. Polymers most useful for hydrogel applications are those that exhibit a lower critical solution temperature (LCST); these polymers behave as a solution below this temperature and as a gel above it [54]. This transition is driven by the development of hydrophobic chain entanglements, which result in phase separation within the aqueous polymer solution as a physically cross-linked network is formed. For biomedical applications, ideal polymers are those that exhibit this transition between room and body temperatures. In this way, the aqueous solution can be injected at room temperature but rapidly transition to a gel at body temperature.

Because this transition is defined by a shift between hydration and hydrophobic interactions governing the polymer's physical state, there are some special considerations for applications to drug delivery. First is that this transition can often result in the expulsion of large amounts of water as the polymer phase separates. For example, poly(*N*-isopropylacrylamide) (PNIPAAm)-based materials heated from 22 °C to 37 °C expel up to 200 wt% of water [55]. This rapid deswelling can drive encapsulated hydrophilic molecules out of the matrix, resulting in a large burst release, although this effect can be modulated by incorporating hydrophilic molecules such as PEG into the polymer matrix [56]. Temperature-sensitive polymers are water soluble at lower temperatures because the hydrogen bonding of the polymers with water is stronger than the hydrophobic interactions between the polymers. As the temperature rises, however, the hydrogen bonding becomes weaker, and the hydrophobic interaction becomes stronger, resulting in precipitation of polymers from solution or collapse of the hydrogel structure. This temperature-dependent increase in hydrophobic character within the hydrogel and/or subsequent network collapse could pose a concern for immunogenicity [57] and antibody stability [58,59], both of which should be carefully examined during investigation of such systems.

In recent years, several ocular protein delivery systems based on temperature-sensitive polymers have been reported. For example, Derwent and Mieler reported in 2008 on a PEG-crosslinked PNIPAAm thermosensitive hydrogel for posterior ocular delivery of proteins [60]. Rapid deswelling of the matrix at 37 °C resulted in a large efflux of the encapsulated protein in the first few hours. In addition, complete release of encapsulated proteins was not observed, indicating some amount was entrapped within the polymer matrix. In 2011, Wang and colleagues reported on a thermosensitive hydrogel that could sustain the release of bevacizumab for approximately 10 days [61]. The material was well tolerated in the vitreous and did not result in physiological changes to the retinal tissues. In 2013, Park and colleagues reported on a reverse thermal gelling polymer intended for intravitreal delivery of bevacizumab [62]. The copolymer system, composed of a tri-block PEGylated polyurethane, was injectable through a 27G needle and transitioned from sol to gel between 32 and 39 °C. Release kinetics of bevacizumab *in vitro* showed a 10–35% burst followed by sustained release over 17 weeks. *In vivo* studies demonstrated an approximately 5-fold improvement in intraocular bioavailability compared to bevacizumab alone, which was sustained for 9 weeks [40].

A novel hybrid system developed by Patel and colleagues entrapped protein-loaded nanoparticles within a thermosensitive gelling polymer [63]. *In vitro* results demonstrated low cytotoxicity and high biocompatibility in cell-based assays, while release of model proteins could be sustained for 60 days with near-zero-order kinetics. Entrapment of nanoparticles within the thermogel was also found to reduce the burst release phenomenon of nanoparticles alone, presumably due to the additional diffusion barrier provided by the hydrogel. One challenge for these types of multicomponent systems is demonstration that sufficient drug can be loaded within 50–100 μL of the formulation to provide a therapeutic level of drug release over the intended duration. In this example, the authors achieved between 5 and 6% drug loading

within the nanoparticle, and it is not clear what duration of clinically meaningful drug exposure this would provide.

Photosensitive hydrogels

That light can be transmitted directly into the posterior segment of the eye in a controlled and targeted manner may open up possibilities for the use of photosensitive or photocrosslinkable hydrogels. As in thermoresponsive polymers, hydrogel precursors could presumably be injected into the vitreous as a solution. However, instead of using temperature to affect gelation, a targeted laser or other light source would be directed at the injected precursor to, e.g. drive crosslinking via a photosensitive crosslinker.

Such a chemistry was demonstrated by Envisia, in which protein microparticles were encapsulated within a photocurable hydrogel [64]. *In vitro*, this system released bevacizumab for 90 days, while its *in vivo* application and performance has yet to be reported. Tyagi *et al.* also employed a light-activated hydrogel system for posterior protein delivery using a suprachoroidal injection of photocurable hydrogel precursors and free bevacizumab [48]. While this system could release the protein *in vivo* for several months, the extended time needed for complete curing resulted in a burst release of more than 20% of loaded protein.

While promising in principle, this approach does carry several potential liabilities for an intravitreally-administered drug delivery system. One such liability is the amount of time required to crosslink the system upon injection and whether this can be done quickly enough to prevent diffusion of precursor components throughout the vitreous. A bigger concern may be the generation of free radicals by photoinitiators. Produced *in situ*, these free radicals may cause toxicity in nearby tissues, which would prohibit their use. Further, the stability of drugs—especially protein therapeutics—would be a major concern in the presence of free radical-generating photoinitiators, as well as after exposure to high intensity UV irradiation.

In situ *forming hydrogels*

Instead of using biological stimuli to initiate gelation after injection, some groups have designed hydrogels that spontaneously form after injection [65]. These *in situ* forming hydrogels form through coupling of reactive species at the injection site. Since there is no stimulus to activate this reaction, these systems are typically formulated such that the various hydrogel precursors are mixed immediately prior to the injection event. The injected pre-gel then spontaneously reacts to form a cohesive network at the site of injection.

In order to design a successful *in situ* gelling protein delivery hydrogel, several key factors must be controlled. First, the rate of reaction must be carefully tuned within a narrow window since network formation must be: a) fast enough to react quickly upon injection to encapsulate the protein and prevent escape of the hydrogel precursors by diffusion away from the injection site; but b) slow enough to allow injection of the pre-gel since viscosity of this solution will increase with network formation and eventually prevent injection.

In situ gelling protein delivery systems have several liabilities that must be considered carefully during the design process [66]. First, diffusion of the protein drug out of the hydrogel before the network is fully formed must be controlled in some way. Strategies to address this will be explored in more detail in Section 5, but may involve immobilization of the protein to the hydrogel precursors in some manner. Second, in order for an *in situ* gelling system to be injectable yet form a network upon injection, some reactive species and/or catalysts must be introduced to the injection site (since stimuli-responsive chemistries are excluded in this category). In order for this to be feasible, reactive chemistries must be carefully chosen that they don't elicit an unwanted reaction *in vivo*. For most intraocular tissues, this bar is relatively high and will exclude most commonly used chemistries for consideration.

In general, the requirements of an *in situ* gelling hydrogel crosslinking chemistry include: a) amenability to reaction in an aqueous physiological environment; b) rapid but controllable kinetics of reaction; c) non-cytotoxicity to surrounding or nearby tissues; and d) non-destructivity to the protein drug being encapsulated within the gel. This relatively demanding list of requirements precludes most commonly used chemistries from serious consideration for a protein-encapsulating hydrogel. However, several more recent chemistries may be able to achieve this, especially those that fall under the category of "bioorthogonal" reactions [67]. These include the copper-free click reaction between azides and cyclooctynes and the tetrazine ligation with norbornene or *trans*-cyclooctene.

Several injectable *in situ* gelling systems have been developed for posterior protein delivery. In 2013, Xu *et al.* reported on an alginate chitosan hydrogel for sustained release of bevacizumab [41]. The release profile was characterized by a 20-30% burst release in the first several hours associated with network formation and complete release within 3 days. In 2014 Yu *et al.* reported on a PEG-based hydrogel formed by the Michael addition reaction between two tetrameric PEG molecules, PEG-maleimide and PEG-SH [68]. Pre-formed gels were found to release bevacizumab over several weeks; however, the release profile of gels formed *in situ* was not included.

Micro- and nanogels

The aforementioned systems seek to overcome the limitation of injectability by designing a system that can transition from a solution to a gel during or after injection. Alternatively, hydrogels can be made injectable by reducing their size to the nano- or micro-scale, which would permit their direct injection into the vitreous or other posterior sites. Hydrogels fabricated at this scale are often referred to as nano- or microgels. This approach is being explored for instance by Envisia Therapeutics, which uses their proprietary PRINT™ technology to fabricate protein-loaded hydrogel microparticles [69].

While overcoming the challenge of injectability, this approach does introduce a new challenge: the greatly reduced distance a protein must diffuse to be released from the hydrogel. As a result, novel release-modifying strategies become especially important to sustain delivery on a usable time scale, as explored in Section 5.

Shear-thinning hydrogels

Shear-thinning hydrogels are another class of injectable hydrogels that takes advantage of transient or reversible interactions instead of covalent bonds to provide crosslink points in the hydrogel network. In the presence of high mechanical shear, such as within a syringe needle during injection, these crosslinks are disrupted

and the hydrogel can flow through the needle. In the absence of shear forces, such as once the hydrogel is deposited within the vitreous, the crosslink points can re-form, forming a cohesive depot [70]. Unlike chemically crosslinked *in situ* gelling systems, where precursors are mixed and covalent bonds are formed after injection, the shear-thinning hydrogel can be formed *ex vivo* because crosslinking is non-covalent. The non-covalent crosslinking of the shear-thinning gel is typically through self-assembly from physical associations such as hydrophobic interactions, hydrogen bonding, electrostatic interactions, and host-guest interactions [71]. Examples of shear-thinning systems are beta-hairpin peptide-based fibrillar hydrogel, recombinant multidomain peptides and cyclodextrin-block copolymer mixtures [70]. A successful shear-thinning system should self-assemble to form the intended network structure at physiological conditions, flow upon injection and self-heal after injection. Kinetics of network recovery after injection and associated drug encapsulation efficiency need to be tuned to ensure shear-thinning hydrogels can be a viable option within a clinical setting.

Drug encapsulation and release strategies

Many sustained drug delivery systems control drug release by limiting the ability of the molecule to diffuse out of the encapsulating matrix. For example, poly(lactic-*co*-glycolic acid) (PLGA) is a relatively dense hydrophobic matrix. The small mesh size characteristic of matrices like PLGA results in small pores and high tortuosity, which are able to limit free diffusion of molecules from the matrix and thereby extend the timeframe over which the drug is released [72]. While these systems have proven effective for controlling the release of small molecule drugs, their hydrophobic nature and relatively extreme manufacturing conditions (i.e. use of organic solvents, high temperature, and/or high shear environment) make them more challenging to implement for delivery of biologics [7].

While hydrogels are better suited to encapsulation of biologics, most have mesh sizes orders of magnitude larger than hydrophobic polymers due in part to the swelling effects of water. As a result, they are not able to effectively limit diffusion of protein molecules out of the matrix, resulting in relatively fast efflux of biologics out of the system (time scales of hours to days are typical for standard macroscopic hydrogel delivery systems). While this time scale may be acceptable for some applications of protein delivery, many require a much longer duration of release to meet the therapeutic requirements.

In order to address this shortcoming, researchers have devised numerous methods of controlling protein efflux to prolong the time scale of release. These include slowing diffusion of the protein through physical or chemical means, among others, and use of permanent or transient linkages between the protein and the hydrogel matrix, as illustrated in Fig. 3.

Diffusion-modulating strategies

The driving force for rapid efflux of proteins from most hydrogels is their largely unencumbered diffusion out of the hydrated hydrogel matrix. By extension, if the diffusion can be encumbered in some way, that should prolong the time required for diffusion out of the hydrogel thereby extending the duration of release. This effect can be achieved by either physical or chemical means, as has been demonstrated in several recent efforts.

The mechanism for physically limiting protein diffusion out of a hydrogel is derived from limiting the effective diffusion coefficient of the molecule through the matrix. This effective diffusion coefficient through a porous medium, D_e, is defined as:

$$D_e = \frac{D\,\varepsilon_t\,\delta}{\tau}$$

where D is the diffusion coefficient of the protein in the medium filling the pores (i.e. water), ε_t is the porosity available for transport, δ is the constrictivity and τ is the tortuosity. Within this equation, porosity and constrictivity (and in practice tortuosity) can all be used to decrease the effective diffusion coefficient by decreasing the mesh size of the hydrogel (the mesh size of a polymer matrix is defined as the average distance between neighboring polymer strands).

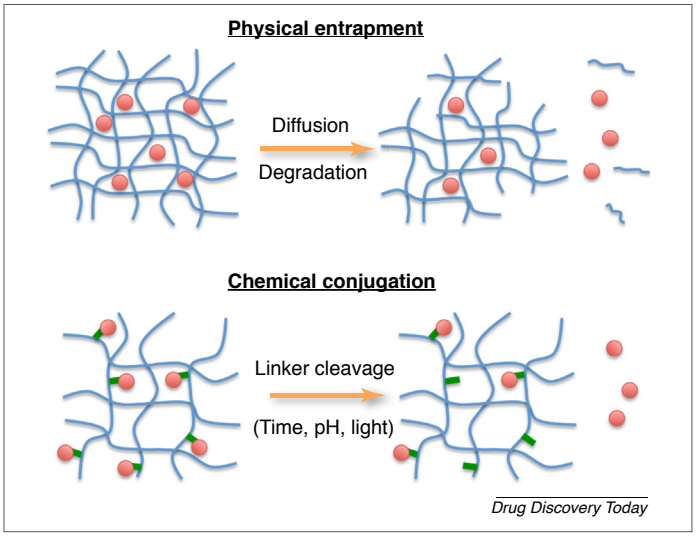

FIGURE 3

Drug encapsulation and release from hydrogel networks can be accomplished by physical entrapment or chemical conjugation. The decision between release control strategies will be dictated by the therapeutic needs of the specific drug being released.

This strategy was employed recently by Tong *et al.* [73] to produce a PEG-based hydrogel that could release a growth factor for approximately two months using BSA as a model protein. In this system, the mesh size of the hydrogel was decreased to be on the order of the hydrodynamic radius of BSA (approximately 4 nm), which effectively immobilized the protein within the PEG network. As the hydrogel network degraded hydrolytically over time, the mesh size increased and the protein could slowly diffuse out. This strategy permitted first-order drug release kinetics for approximately 60 days using BSA as a model.

An important note in this approach is the requirement that the protein be present during the hydrogel formation process since the small mesh size won't allow the protein to enter the network once it is formed. As a result, the crosslinking or polymerization chemistry used must be non-degrading to the protein. Commonly used photo-initiating species, such as those used by Tong *et al.*, are likely to chemically degrade proteins or attach them to the polymer network [74]. These details are often overlooked in published reports, but require careful attention from investigators to preserve the biological availability and activity of released proteins.

An alternative strategy employed by Ocular Therapeutix has been to encapsulate the protein within a relatively small particle, which is then entrapped within a hydrogel matrix [75]. Since the protein is not free to diffuse within the hydrogel, its release is dictated by degradation of the hydrogel matrix and can be tuned as such. This strategy—encapsulation of the protein within a matrix, which is dispersed within a hydrogel—has also been explored by other groups, e.g. using PLGA particles [76–78].

In addition to physical mechanisms of preventing free protein diffusion through a hydrogel, electrostatic interactions have also been used. In these hydrogels, charged groups are introduced such that the hydrogel can electrostatically interact with the protein. If these groups have charges opposite the charge on the protein at physiological pH, the attractive forces resulting from this interaction can slow diffusion of the protein out of the matrix. For example, Purcell *et al.* incorporated sulfated hyaluronic acid (HA) into HA gels to slow release of a highly positively charged model protein [79]. Compared to the more neutral albumin, whose release was not modified by incorporation of negative sulfate groups, release of the positively charged protein was approximately 3-fold slower when sulfate groups were present.

From a practical standpoint, this approach is less broadly applicable than other strategies since it relies entirely on the isoelectric point of the protein of interest. If the target protein isn't sufficiently positively or negatively charged at physiological pH, ionic interactions won't be strong enough to provide a controlling effect on their diffusion. In addition, the presence of salts in physiological environments can screen this interaction, thereby reducing its effect *in vivo*. For these reasons, this strategy may be challenging to practically implement.

Chemical conjugation

The strategies described in the previous section to limit free diffusion of proteins have been used in research fairly extensively, but their translation to a clinical product has been slow due to the numerous challenges described. In response to the challenges presented by those approaches, researchers began exploring the idea of chemically attaching the protein to the hydrogel backbone in a reversible manner. The linker between the protein and the hydrogel was made responsive to physiological stimulus (e.g. pH) to release the protein at the desired rate largely independent of the hydrogel properties.

This cleavable linker strategy is an area of active development with several companies exploring this space. ProLynx, LLC. and Ascendis Pharma are two examples of companies developing linkers for drug delivery that can transiently attach a protein to a hydrogel backbone and release the free drug over an extended time under physiological conditions [42,80,81]. Critically, this cleavable linker strategy liberates several hydrogel properties from being dictated by protein release behavior. For example, since diffusion of the protein isn't a critical parameter in determining its release behavior, hydrogels could be fashioned as nano- or microparticles with minimal impact on the release kinetics. In addition, mesh size, swelling and degradation properties can be decoupled from release kinetics, further freeing up formulation flexibility. For these reasons, this approach may have a significant impact on the development and translation of future ocular protein delivery hydrogels.

Development considerations

To bring any ocular delivery technology to market, it is of paramount importance that the delivery vehicle is safe and that the production is tightly controlled. Safety must be demonstrated in pre-clinical animal testing before an investigational new drug application (IND) is submitted and approved for clinical testing. The production of the drug delivery system must be tightly controlled so that it is consistent from batch to batch. These activities are known as chemistry, manufacturing and control (CMC). The following sections discuss some of the challenges that will need to be considered for development of a successful ocular drug delivery system.

Clinical considerations

One of the most important requirements for any ocular delivery technology is pre-clinical demonstration of safety and tolerability of the entire delivery system, including the procedure of administration. The retina in particular is a complex tissue in which minor perturbations can adversely affect vision; therefore, a complete lack of retinal toxicity is paramount. Severe and/or sustained adverse reactions can lead to irreversible damage, e.g. retinal detachment. Inflammation and foreign body reactions in all ocular tissues need to be quite limited or non-existent, as demonstrated by appropriately designed pre-clinical safety studies.

An important design aspect of any hydrogel intended for ocular use is clearance of the gel from the site of injection, which should ideally occur in a similar time frame to drug release. Since most back of the eye diseases are chronic and would require continuous re-administration of the drug depot, accumulation of hydrogel components over time must be prevented. This can be achieved through selection of a biodegradable polymer backbone, incorporation of degradable crosslinkers, or through engineered disassembly of the hydrogel network over time (e.g. as is often the case for shear-thinning hydrogel formulations). Relatedly, demonstration of safety and tolerability of the injected hydrogel should be paired with an assessment that the degradation products resulting from hydrogel erosion or disassembly are also well tolerated.

The intended form of the hydrogel formulation will also play a critical role in defining associated pre-clinical testing requirements. As most hydrogels are cross-linked polymer networks, a strategy for delivery must be established early in pre-clinical testing. The injection force, modeled by the Poiseuille equation, is inversely proportional to the fourth power of the needle radius and directly proportional to the viscosity, injection speed and square of syringe plunger radius [46]. To reduce the injection force, the hydrogel solution viscosity must be low or be lowered during injection, for example through use of a thermosensitive, shear-thinning or *in situ* forming chemistry. In all of these cases, pre-clinical testing should be used to confirm reproducible recovery of hydrogel properties after injection. Alternatively, hydrogels may be constructed as nano- or micro-gels to facilitate injection into the eye. Based on previous reports, careful attention must be given to the fate of injected particles as there is evidence that such particles are mobile and can migrate outside of the intended tissue [82]. For *in situ* forming hydrogels that crosslink upon injection, the mixing of the gel precursors and the time to cohesive gelation are two important factors that need to be evaluated before advancement to clinical testing.

Chemistry, Manufacturing and Controls

All aspects of the chemistry and manufacturing process of the drug delivery system need to be well defined with appropriate, validated analytical methods to monitor the product. The International Conference on Harmonization (ICH) has set guidelines on the specifications, test procedures and acceptance criteria for new drug substances and drug products for small molecules and biologics. Specifications are part of a control strategy to ensure product quality and consistency. For a novel drug delivery system, new test procedures need to be established and validated. For hydrogels derived from natural polymers such as alginate or hyaluronic acid, raw material specifications and thorough characterization of impurities will be critically important to ensure proper performance and control.

Manufacturability of the hydrogel delivery system is another important challenge. Any laboratory scale synthesis will need to be scaled up and all the handling and processing will need to be evaluated. If the drug needs to be chemically conjugated to the hydrogel, then the conjugation process and scalability will need to be addressed. The protein therapeutic needs to be stable during the conjugation process and at the same time any chemicals used during the synthesis step will need to be removed, as any residual materials may affect tolerability in the eye.

For all ophthalmic delivery systems, the United States Pharmacopeia (USP) has set guidelines that must be followed. The monograph Ophthalmic Preparations – Quality Tests <771> describes the quality tests and that should be applied [83]. Hydrogels are described as novel ophthalmic dosage forms, which must follow the general ophthalmic delivery guidelines. Ophthalmic drug delivery products must meet specifications for all general quality attributes such as identity, purity, potency, sterility and particulate matter. In addition, any drug-releasing system will need controls and assays in place to evaluate lot-to-lot drug release performance and variability. Establishment of accelerated release and/or degradation assays early in the development process may greatly facilitate development activities.

Sterility, bioburden and pyrogenic impurity testing are critical aspects of drug product quality testing. For hydrogel delivery systems, any sterilization process must not induce degradation or morphological changes to the hydrogel or drug components. Depending on the hydrogel system, autoclaving at elevated temperature may be an option, but protein stability will likely be adversely affected through this harsh process. Sterile filtration is only applicable to formulations with particulates less than 0.2 μm in diameter. Many cross-linked hydrogels with conjugated or encapsulated biologics will require an aseptic manufacturing process after sterile filtration of liquid components and sterilization of other process inputs to achieve a sterile drug product.

Information on all aspects of the CMC process will be required for submission to FDA to ensure proper identity, quality, strength/potency, and purity of the drug substance and drug product. Physicochemical characterization of the hydrogel drug product such as morphology, size, viscosity and *in vitro*/*in vivo* degradation will be critically useful for regulatory filings. Stability studies of the hydrogel drug product will be needed to establish storage conditions and shelf life. Development of any ocular drug delivery system will be a technical and clinical challenge, but it could have a significant impact in improving treatment for many patients.

Conclusions

Over the past decades, there have been considerable developments and advances in ocular macromolecular therapeutics. Anti-VEGF therapies for wet AMD set the stage for development of more macromolecule therapeutics treating back of the eye diseases. Many such therapeutics are currently in development, increasing the need for innovative ocular delivery methods. Sustained delivery of biologics with hydrogels has a promising future because of the highly biocompatible and protein-compatible nature of many hydrogel chemistries. The physiochemical properties of the hydrogel can be engineered to respond to external stimuli such as pH, temperature and light. These stimulus-responsive properties can be used to make hydrogels injectable and allow for localized delivery to the back of the eye. Drug encapsulation and release strategies for hydrogel delivery systems include physical entrapment and chemical conjugation. The major challenge now is to design a hydrogel system that can provide sustained drug release for several months and is completely physically cleared at the end of drug release, or relatively soon thereafter, to avoid matrix accumulation. The future for hydrogel drug delivery systems is promising, but advances must be made through interdisciplinary collaborations to address some of the important challenges such as clinical translation, safety and tolerability, drug stability, and manufacturability. Overcoming these remaining hurdles will allow hydrogel ocular drug delivery systems to realize their full potential to treat otherwise debilitating ocular diseases while reducing the burden of treatment for patients and clinicians.

Conflicts of Interest

The authors declare that there are no competing financial interests.

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