



REVIEW

Ocular delivery systems for the administration of antibody therapeutics

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Abstract Antibodies (Abs) have been extensively used as a powerful tool for targeting proteins based on their immunologic functions such as antigen recognition and pathogen neutralization. In particular, monoclonal Abs (mAbs) and fragments can be promising drugs in clinics owing to their sensitivity and specificity. The controlled release of Ab drugs after local delivery would lead to their prolonged exposure at the disease site, thereby improving the disease condition. In this review, we illustrate the activity of clinically used anti-vascular endothelial growth factor drugs, including aflibercept (Eylea; Regeneron Pharmaceuticals, Bayer Pharma), bevacizumab (Avastin; Genentech, Novartis), and ranibizumab (Lucentis; Genentech, Novartis), in ocular diseases such as wet age-related macular degeneration and myopic choroidal neovascularization. For controlled and prolonged release of the aforementioned drugs after ocular administration, recent approaches using liposomes, hydrogels, and nanoparticles have been introduced. In addition, the evaluation methods to meet the requirements of clinically used Abs are discussed. On the basis of the findings of our review, we can suggest that an ocular delivery system can be a promising platform to overcome the limitations associated with clinically used Abs.

Keywords Ocular delivery system · Antibody therapeutics · Anti-vascular endothelial growth factor · Ocular disease · Controlled release

Introduction

Although antibodies (Abs) possibly induce hypersensitivity or allergic reactions, they are one of the powerful strategies to improve a disease condition in humans. After recognizing and binding to specific antigens, Abs trigger various biological functions (Volz and Pauly 2015). Therapeutic Abs are extensively used in clinics for improving immunologic and oncologic responses in patients (Chinen et al. 2015; Lao et al. 2015). The first Ab drug approved by the Food and Drug Administration (FDA) was a murine-derived muromonab-CD3 (Orthoclone OKT3), a CD3 monoclonal antibody (mAb) for acute organ rejection of organ transplants, in 1986 (Parlevliet and Schellekens 1992). Many Ab drug candidates are currently undergoing clinical trials (Reichert et al. 2005).

Based on their structural characteristics, Ab drugs exert different functions via interaction with disease-induced proteins, Ab-dependent cellular cytotoxicity, and complement-dependent cytotoxicity (Elgundi et al. in press; Weiner 2015). In general, the Y-shaped structure of immunoglobulin G (IgG; Fig. 1) can be divided into fragment antigen-binding region (Fab) and fragment crystallizable region (Fc). Each IgG molecule consists of four polypeptide chains: two identical heavy chains (H) and two identical light chains (L). These chains are made up of variable regions (V), which contain variable amino acid terminals in Fab, and constant (c) regions. Thus, there is one variable domain (V_H) and three constant domains (C_H1, C_H2, and C_H3) in the H chain, and one variable domain (V_L) and one

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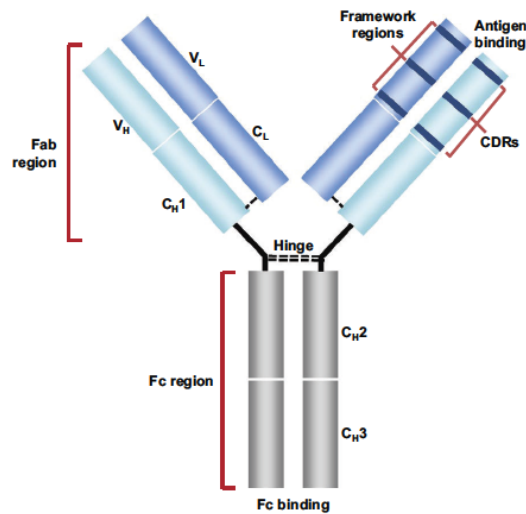


Fig. 1 Schematic diagram of Ab structure: immunoglobulin G (IgG)

constant domain (C_L) in the L chain. The H and L chains of the Ab are linked together by non-covalent interactions and covalent interchain disulfide linkages at the hinge region. The complementarity-determining regions (CDRs), also called hypervariable domains, are hypervariable regions present in the variable domains (V_L and V_H). There are three pairs of non-identical CDRs (CDR1, CDR2, and CDR3), and they are responsible for antigen specificity. Since mAbs are monospecific and bind to specific cells or proteins of the target, they have a promising potential as a drug (Weiner 2015). Recently, the fragments in Abs such as Fabs, second-generation single-chain variable fragments (scFvs), and third-generation (3G) Abs have also been studied (Elgundi et al. in press; Khalili et al. 2013; Nelson and Reichert 2009).

Local administration of clinically used Abs requires lower doses than systemic administration, which in turn results in reduced systemic exposure and enhanced efficacy (Davis et al. 2014; Dickmann 2016). Applying lower doses of Ab drugs helps to minimize the side effects of Abs in the body (Agrahari et al. 2016; Burt et al. 2016). In ophthalmology, for the treatment of ocular diseases such as wet age-related macular degeneration (AMD) and myopic choroidal neovascularization (CNV) (Wong et al. 2015), anti-vascular endothelial growth factor (VEGF, 36–46 kDa) drugs are routinely administered via direct intravitreal injection into the eyes (Bolinger and Antonetti 2016; Kim et al. 2014; Volz and Pauly 2015; Zhang et al. 2012). Although bevacizumab (149 kDa, Avastin; Genentech, Novartis) was originally applied via intravenous infusion,

when used for ocular treatment, an intravitreal injection is used to facilitate its local delivery (Schmid et al. 2015; de Oliveira Dias et al. 2016). Ranibizumab (48 kDa, Lucentis; Genentech, Novartis), the first Ab drug approved by FDA for localized therapy via intravitreal injection, is a fragment of VEGF Ab (Network 2015). Moreover, aflibercept (97 kDa, Eylea; Regeneron Pharmaceuticals, Bayer Pharma), a fusion protein, is a locally applied VEGF inhibitor (Schmid et al. 2015). To overcome the limitations associated with delivery of Ab therapeutics via different administration routes, localized and targeted delivery systems of Abs can be applied for prolonged exposure of the drugs at the disease site (Kim et al. 2014; Schweizer et al. 2013).

In this review, we highlight three representative Ab drugs, aflibercept, bevacizumab, and ranibizumab, for the treatment of various ocular diseases including their off-label uses investigated by clinicians. We also focus on localized targeted systems via different administration routes for effective Ab therapy. Liposomes, hydrogels, and nanoparticles are introduced as delivery systems for application strategies involving the controlled and prolonged release of Abs. Finally, the requirements for Abs to be considered for therapeutic purpose and their evaluation criteria are discussed. From this review, scientists as well as clinicians can expand the clinical scope of localized and targeted delivery systems for Ab therapeutics.

Representative Ab therapeutics in ocular diseases

We discuss about the representative Ab drugs of aflibercept, bevacizumab, and ranibizumab administered via intravitreal injection for the treatment of ocular diseases (Radhakrishnan et al. 2017). The characteristics of aflibercept, bevacizumab, and ranibizumab used for the treatment of wet AMD, central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO), diabetic macular edema (DME), and myopic CNV, including an off-label use is listed in Table 1.

Ocular diseases

Ocular diseases affect the quality of life in patients owing to significant visual impairment. Wet AMD, CRVO, BRVO, DME, and myopic CNV are ocular diseases that lead to vision loss (Barar et al. 2016). Dry AMD progresses to wet AMD owing to ocular ischemia, which results in unwanted neovascularization of macula followed by macular degeneration (Laude et al. 2010; Schmid et al. 2015; Volz and Pauly 2015). CRVO is also a leading cause of vision loss due to macular edema along with macular ischemia (Claudio et al. 2016; Hayreh 2014). However, BRVO is a retinal vascular disorder characterized by intraretinal hemorrhage,

Table 1 Representative Ab therapeutics in clinics for the treatment of ocular diseases

Agents (trade name; company)	Target	Ab types	Mechanism of action	Molecular weight (KD)	Route of administration	Ocular diseases	Refs.
Aflibercept (Eylea; Regeneron Pharmaceuticals, Bayer Pharma)	VEGF and PlGF	Fusion protein (IgG1 + Fc)	Anti-angiogenesis	97 (protein), 115 (total)	Intravitreal injection	Wet AMD, CRVO, BRVO, DME, myopic CNV	Schmid et al. (2015), de Oliveira Dias et al. (2016)
Bevacizumab (Avastin; Genentech, Novartis)	VEGF-A	Monoclonal IgG	Anti-angiogenesis	149	Intravitreal injection	Wet AMD, CRVO, BRVO, DME, myopic CNV	Schmid et al. (2015), Malik et al. (2014)
Ranibizumab (Lucentis; Genentech, Novartis)	VEGF-A	Fab-fragment	Anti-angiogenesis	48	Intravitreal injection	Wet AMD, CRVO, BRVO, DME, myopic CNV	Chong (2016), Network (2015)

VEGF vascular endothelial growth factor, PlGF placental growth factor, Wet AMD neovascular (wet) age-related macular degeneration, CRVO macular edema following central retinal vein occlusion, BRVO macular edema following branch retinal vein occlusion, DME diabetic macular edema, myopic CNV myopic choroidal neovascularization

retinal ischemia, and macular edema (Claudio et al. 2016; Hayreh 2014). The main factor inducing vision loss in BRVO is the presence of fluid in the macula, known as macular edema. DME is an advanced vision-limiting complication of diabetic retinopathy (Boling and Antonetti 2016). It is mediated by retinal ischemia, which leads to the breakdown of the inner blood retinal barrier and vascular leakage. Myopic CNV is a common complication of pathological myopia that progresses to macular atrophy, which in turn leads to irreversible vision loss (Teo et al. 2016). In all the above-mentioned diseases, vision loss was caused by overexpression of VEGF and mediated by blood vessel proliferation. VEGF is a glycoprotein that enhances angiogenesis and vascular permeability (Stefano et al. 2012). Among the VEGF isoforms, VEGF-A isoforms support endothelial cell proliferation and survival, which is mediated by degradation of basement membrane and stimulation of cell migration. Therefore, anti-VEGF therapy has been used to improve disease conditions.

Anti-VEGF drugs

Anti-VEGF therapy is the administration of VEGF-binding molecules to neutralize the downstream signaling of VEGF. Abs of VEGF have been administered to local disease sites of the eye via intravitreal injection (Radhakrishnan et al. 2017). Aflibercept, bevacizumab, and ranibizumab target VEGF, specifically VEGF-A isoforms, for the inhibition of angiogenesis in wet AMD, CRVO, BRVO, DME, and myopic CNV (Radhakrishnan et al. 2017; Kim et al. 2014). Abs bind to VEGF-A isoforms to prevent the binding of these isoforms to VEGF receptor-1 (VEGFR-1) and VEGF receptor-2 (VEGFR-2), as well as to block VEGF-A

isoform-mediated angiogenesis. Three Ab drugs have different structures with different binding affinities to VEGF-A isoforms (Fig. 2). Aflibercept (Fig. 2a) is a fusion protein having a binding affinity to VEGF-A isoforms, VEGF-B isoforms, and placental growth factor (PlGF), whereas

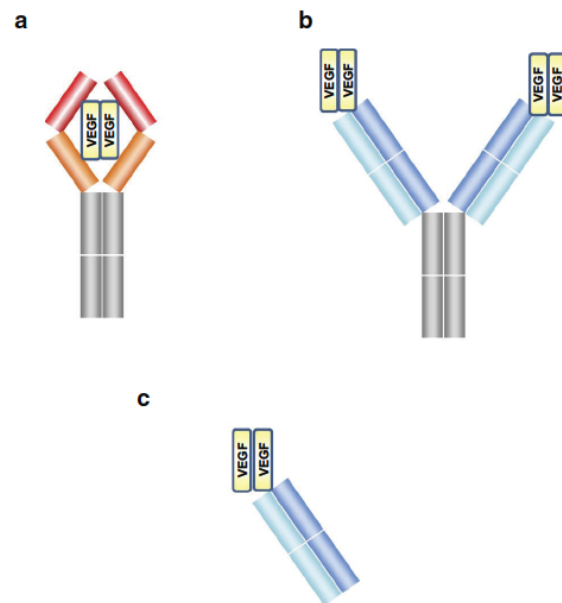


Fig. 2 Schematic diagrams of the structures of Anti-VEGF drugs in ocular diseases: **a** aflibercept, **b** bevacizumab, and **c** ranibizumab. Aflibercept is a fusion protein with a VEGF-A and VEGF-B binding trap, showing higher binding activity than ranibizumab and bevacizumab. Bevacizumab is a VEGF mAb with VEGF-A binding activity. Ranibizumab is an Ab fragment with VEGF-A binding activity

bevacizumab (Fig. 2b) is a humanized mAb that binds to VEGF-A isoforms. Ranibizumab (Fig. 2c) is a small affinity short lived Fab fragment that binds to VEGF-A isoforms. It is administered at a dose of 0.5 mg/month (Chong 2016).

Eye as an administration route

Eye is an alternative delivery route for localized therapy in ocular diseases. For ocular diseases, application techniques should be considered at the target sites in the eye (Kim et al. 2014; Osswald and Kang-Mieler 2016). The eye structure and various administration routes with application techniques from eye drop to intravitreal injection are shown in Fig. 3. In this section, we discuss the eye structure and administration techniques in ocular diseases.

Eye structure

The eye is divided into the anterior and the posterior segments (Fig. 3). The anterior segment comprises the cornea, conjunctiva, ciliary body, iris, aqueous humor, lens, and lachrymal system. The posterior segment consists of the retina, vitreous humor, sclera, and choroid. The main structural barriers for drug application to the eye are (1) the cornea and anterior barriers, (2) the sclera and choriocapillaris,

and (3) the retina and blood-retinal barrier (Mandal et al. 2017).

The cornea is a transparent tissue arranged in five layers in the front of eye, and it is continuous with the sclera. It is a primary barrier of topical drug absorption. Since the cornea is negatively charged, positively charged materials exhibit better corneal permeability (Kim et al. 2014; Rabinovich-Guilatt et al. 2004). The conjunctiva is a thin translucent layer that covers the sclera. The conjunctival epithelium has tight junctions that prevent the penetration of molecules. In the conjunctiva, the drug molecules can be absorbed to the intracellular spaces and finally into the systemic circulation through the blood and lymphatic vasculatures. The intraocular environment, including the aqueous humor, is protected by the blood-aqueous barrier, which regulates active and paracellular transport. Dextran (150 kDa) can penetrate this route (Bellhorn 1981; Kim et al. 2014). The sclera and choriocapillaris also serve as absorption barriers of macromolecules. Macromolecules can penetrate the sclera and choriocapillaris, but the permeability drops significantly at high molecular weight. The retina is a photosensitive layer that acts as a diffusion barrier to macromolecules. The blood-retinal barrier is divided into inner and outer barriers. The inner and outer blood retinal barriers depend on the integrity of tight junctions between the retinal endothelial vasculatures and retinal pigment epithelium, respectively.

Wet AMD is characterized by neovascularization of Bruch's membrane, which is the innermost layer of the choroid. This neovascularization results in the invasion of subretinal tissues due to increased VEGF secretion in the retinal pigmented epithelium and oxidative stress (Volz and Pauly 2015). Leakage of fluid and blood within the tissues causes edema, resulting in rapid cell death and vision loss. In ocular diseases such as wet AMD and myopic CNV, Abs can diffuse into the vitreous compartment driven by a concentration gradient from the injection site or from delivery systems like gels, when injected intravitreally. In such cases, vitreous humor can also be a diffusion barrier. It fills the space between the retina and lens, comprising 80% of the eye, and consists of collagen and glycosaminoglycans (Laude et al. 2010; del Amo et al. 2017). Drug diffusion into the vitreous humor is dependent on the molecular weight of the drug. Small molecular drugs such as ganciclovir (255 Da) showed a short half-life (7.1 h) in rabbit model after intravitreal injection (Lopez-Cortes et al. 2001). However, bevacizumab (149 kDa) had a prolonged half-life (3–4 days) in rabbit models (Bakri et al. 2007).

Rabbits were extensively used as animal models to screen drug efficacy and pharmacokinetics in ocular diseases including wet AMD (Vaquer et al. 2013). Compared to humans, the rabbit model has a smaller vitreous chamber and shorter vitreous diffusional path length (vitreous humor

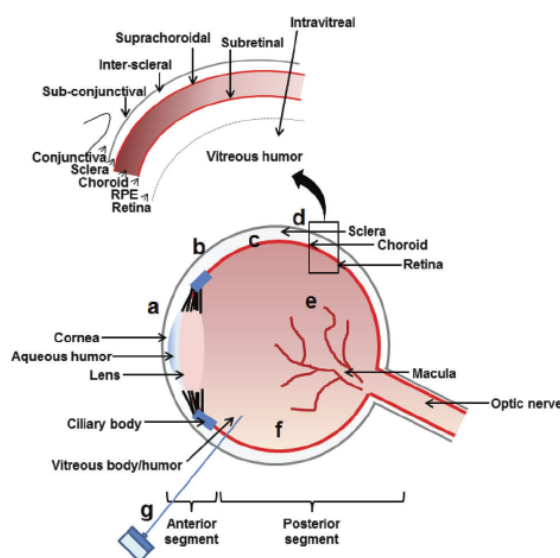


Fig. 3 Schematic diagrams of eye structure and administration techniques: *a* eye drop, *b* scleral plug, *c* subconjunctival implant, *d* suprachoroidal implant, *e* suprascleral injection, *f* intravitreal implant, and *g* intravitreal injection

of human, weight: 3.9 g, volume: 3.9–5.0 mL, vitreous path length: 1.65 cm, and 2.5–3.0 $\mu\text{L}/\text{min}$; vitreous humor of rabbit, weight: 1.4–1.7 g, volume: 1 mL, vitreous path length: 0.78 cm, and 3.6 $\mu\text{L}/\text{min}$) (Laude et al. 2010). Vitreous diffusional path length is defined as the distance from the injection site to the macula, and it is related to drug clearance, predicting higher clearance in small animals.

Application methods

Various techniques such as eye drops and injections have been extensively used for the application of drugs into the eye (Fig. 3) (Grossniklaus et al. 2010). Extraocular delivery of drugs is used for the treatment of ocular diseases in the external, corneal, and anterior segments. In the case of extraocular delivery, drugs can be topically applied using an eye drop. Eye drops (Fig. 3a) are the most popular dosage form in ophthalmic delivery systems because it is non-invasive and easy to handle (Achouri et al. 2013). However, macromolecules in the eye drop show a low bioavailability owing to their restricted absorption and rapid clearance caused by limited permeability and residence time of drugs. Thus, other routes are needed to apply macromolecules into the eye. The intraocular route is necessary to bypass the ocular barriers for delivering Ab therapeutics to the eye (Kim et al. 2014). In addition, administration of Abs via intraocular routes has advantages of prolonging the residence time and reducing the distance of the diffused drug from the matrix and target. For intraocular routes, sclera plug (Fig. 3b), subconjunctival implant (Fig. 3c), suprachoroidal implant (Fig. 3d), suprascleral injection (Fig. 3e), intravitreal implant (Fig. 3f), and intravitreal injection (Fig. 3g) are used to administer Abs in diseases of the posterior segment of the eye (Mandal et al. 2017).

Among the different application methods via intraocular routes, anti-VEGF drugs, aflibercept, bevacizumab, and ranibizumab are intravitreally injected into the eye approximately once a month (Malik et al. 2014; Network 2015; Radhakrishnan et al. 2017; Schmid et al. 2015). Intravitreal injection is a generally used, invasive technique to deliver drugs into the intravitreal cavity in the center of the eye (Radhakrishnan et al. 2017). The normal volume of intravitreal injection that does not affect vision is in the range of 20–100 μL . Krohne et al. monitored the ranibizumab concentration in aqueous humor after a single intravitreal injection of 0.5 mg (Krohne et al. 2012). Ranibizumab in the aqueous humor remained in the range of 36.9–66.1 $\mu\text{g}/\text{mL}$ on the first day after injection. The half-life of ranibizumab was 7.2 days, which was lower than that of bevacizumab (9.8 days). Saunders et al. reported the determination of VEGF-A bound to ranibizumab following intravitreal injection in patients with wet AMD (Saunders et al. 2015). A single dose of ranibizumab at 0.5 mg would

lead to a concentration of approximately 2 μM at an initial intraocular region. The ocular pharmacokinetic profile of VEGF-A bound to ranibizumab showed that the duration of VEGF-A suppression in the aqueous humor and the elimination half-life were 41 ± 9.5 days (28–67 days) and 3.5 days (2.3–5.5 days), respectively. Muether et al. also reported that the time period for complete VEGF-A suppression in patients with AMD was 36.4 ± 6.7 days (26–69 days) (Muether et al. 2013).

Ocular delivery systems

Ocular delivery systems of Ab therapeutics are extensively studied and developed to overcome the limitations of conventional administration systems (e.g., poor bioavailability, rapid clearance, potential toxicity of drugs, and the complications of endophthalmitis, retinal detachment, iritis, uveitis, intraocular hemorrhage, cataract, and hypotony by repeated injections) (Zelikin et al. 2016; Baranowski et al. 2014). Representative systems such as liposomes, hydrogels, and nanoparticles (Fig. 4) are used for prolonged and extended residence time of ocular therapeutics at disease sites, thereby minimizing the frequency of invasive administration (del Amo et al. 2017; Diebold and Calonge 2010). The recently developed ocular delivery systems, along with their compositions, models, and pharmacokinetic/pharmacodynamic (PK/PD) effect are presented in Table 2.

Liposomes

Liposomes (Fig. 4a) are biocompatible and biodegradable systems composed of a phospholipid bilayer that mimics the constituents of the cell membrane. They are spherical in shape, with nanoscale size and minimal cytotoxicity. Liposomes enhance the residence time of drugs at the target site and minimize the adverse response of drugs. They have already been used for the intraocular delivery of small molecules such as brinzolamide (Li et al. 2016) and amphotericin B (Bae and Lee 2015). Liposomal hybrid system coated with polymers of chitosan (Tan et al. 2017) and in situ deacetylated gellan gum gels (Yu et al. 2015) for timolol maleate were studied for use as an eye drop to improve the PK parameters. Davis et al. reported the ocular delivery of Ab therapeutics using annexin A5-associated liposomes containing bevacizumab (Davis et al. 2014). In rat and rabbit models, bevacizumab-loaded annexin A5-associated liposomes penetrated the corneal epithelial barriers after topical application. After penetration of bevacizumab into the eye, bevacizumab-loaded annexin A5-associated liposomes showed an enhanced in vivo delivery potential and reached physiologically significant concentrations at the posterior segment of rats (127 ng/g)

Table 2 Recent approaches of ocular drug delivery systems for Ab therapeutics

Delivery systems	Description	Ab drugs	In vitro/in vivo (administration route)	Model	PK/PD effect	Refs.
Liposome	Annexin A5-associated liposomes	Bevacizumab	In vivo (topical delivery)	Rat and rabbit	Enhanced delivery potential across biological barriers	Davis et al. (2014)
	Nanoliposomes	Bevacizumab	In vivo (intravitreal injection)	Rabbit	Prolonged residency	Abrishami et al. (2009)
Hydrogel	PEG-PSHMu thermoresponsive hydrogel	Bevacizumab	In vivo (intravitreal injection)	Rabbit	Sustained release	Rauk et al. (2014)
	PEG-DA cross-linked PNI-PAAm thermoresponsive hydrogel	Bevacizumab and ranibizumab	In vivo (intravitreal injection)	Rat	Extended release	Kang and Mieler (2008)
	PEOz-PCL-PEOz triblock copolymer thermoresponsive gel	Bevacizumab	In vivo (intravitreal injection)	Rabbit	Extended release	Wang et al. (2012)
	PLGA-PEG-PLGA thermoresponsive hydrogel	Bevacizumab	In vivo (intravitreal injection)	Rat	Sustained release	Xie et al. (2015)
	PLGA microsphere—PNI-PAAm-based thermoresponsive hydrogel	Ranibizumab and aflibercept	In vitro	Release medium (phosphate buffered saline) and human umbilical vascular endothelial cells (HUVECs)	Controlled release	Osswald and Kang-Mieler (2016)
Nanoparticle	Light-activated, in situ forming PCM—HEMA gel	Bevacizumab	In vitro (suprachoroidal injection)	Rabbit and rat	Sustained release	Tyagi et al. (2013)
	Albuminated PLGA nanoparticles	Bevacizumab	In vivo (intravitreal injection)	Rabbit (CNV)	Controlled-release	Varshochian et al. (2015)
	Albumin containing PLGA nanoparticles	Bevacizumab	Ex vivo (intravitreal injection)	Rabbit	Prolonged release	Varshochian et al. (2013)

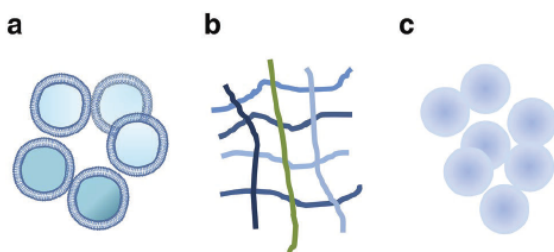


Fig. 4 Schematic diagrams of ocular drug delivery systems for Ab therapeutics: **a** liposome, **b** hydrogel, and **c** nanoparticles

and the retina of rabbits (18 ng/g), suggesting annexin A5-mediated endocytosis. Abrishami et al. also reported that nanoliposome-based bevacizumab prolonged the residence time of bevacizumab in a rabbit model after intravitreal injection (Abrishami et al. 2009). Compared to free bevacizumab, nanoliposomes elevated the bevacizumab concentrations in vitreous samples of rabbits at 28 days (free bevacizumab, 28 $\mu\text{g/mL}$; nanoliposome, 48 $\mu\text{g/mL}$) and 42 days (free bevacizumab, 3.3 $\mu\text{g/mL}$; nanoliposome, 16 $\mu\text{g/mL}$) after administration.

Hydrogels

Hydrogels (Fig. 4b) can be defined as a three dimensional cross-linked macromolecular network of hydrophilic polymers expanded by water. They are a representative delivery system for prolonging the residence time and facilitating the controlled release of Abs at the target site. It can be used as an injectable system for macromolecules because of the poor permeability of macromolecules into the eye. Specifically, *in situ* gel is preferred for the application of Abs via ocular route owing to its ease of administration. In this case, hydrogel is formed *in situ*, within the body, after the injection of a low viscosity liquid, which undergoes sol–gel transition in response to stimulation (e.g., temperature, pH, electric field). For example, thermosensitive gels are based on polyethylene glycol (PEG)-polyserinol hexamethylene urethane (PSHMU) (Rauck et al. 2014), polyethylene glycol-diacrylate (PEG-DA) cross-linked poly N-isopropylacrylamide (PNIPAAm) (Kang and Mieler 2008), poly(2-ethyl-2-oxazoline)-b-poly(ϵ -caprolactone)-b-poly(2-ethyl-2-oxazoline) (PEOz-PCL-PEOz) triblock copolymer (Wang et al. 2012), polylactic-co-glycolic acid-polyethylene glycol-poly(lactic-co-glycolic acid) (PLGA-PEG-PLGA), and PLGA microsphere-PNIPAAm (Osswald and Kang-Mieler 2016). Polycaprolactone dimethacrylate (PCM)-hydroxyethyl methacrylate (HEMA) gel is generated by ultraviolet activation with 2, 2-dimethoxy-2-phenylacetophenone (DMPA) as a photoinitiator (Tyagi et al. 2013). While using *in situ* gel-forming agents, the toxicity

and adverse response of the additives used as stimulators should be evaluated after sol–gel transition. After intravitreal injection, the applied Abs are distributed in the vitreous cavity; they enter into the target cells in the posterior segment and remain there up to several months. Osswald et al. reported the controlled and extended release of ranibizumab and aflibercept *in vitro* for 196 days (6.5 months) (Osswald and Kang-Mieler 2016). They used PLGA-PNIPAAm thermoresponsive hydrogel system, which showed no cytotoxicity in human umbilical vascular endothelial cells (HUVECs).

Nanoparticles

Nanoparticles (Fig. 4c) are one of the popular platforms for nano-sized particles and the delivery of macromolecules *in vitro* and *in vivo*. It shows no cytotoxicity at the dosing level of nanoparticle-based drugs using biocompatible and biodegradable nanomaterials such as albumin and PLGA (Diebold and Calonge 2010). Albumin is an abundant protein in blood plasma, which can be used as a versatile delivery system owing to its targeting potential to specific tissues and potential to extend the half-life after drug administration. In particular, Fuchs and Igney insisted that ocular albumin can extend half-life of intravitreally injected drugs, allowing for longer dosing intervals (Fuchs and Igney 2017). Albumin-based delivery systems like paclitaxel (Abraxane, Celgene) using nanoparticle albumin-bound (nab) technology are already used in clinics to improve safety and efficacy of drugs. PLGA is a synthetic biodegradable polymer approved by the FDA. It can be a controlled release matrix system of Abs based on the regulation of degradation rates by hydrolysis. PLGA is also used in commercially available products of leuprolide (Lupron depot, Abbvie). Albuminated PLGA nanoparticle systems were intravitreally injected for the controlled delivery of bevacizumab (Varshochian et al. 2013, 2015). Varshochian et al. reported improved responses of bevacizumab on PK/PD and pharmacological aspects in rabbit model (Varshochian et al. 2015). Bevacizumab from albuminated PLGA nanoparticles was detected at the posterior segment with 3.3-fold increase for 8 weeks compared with the control group (Varshochian et al. 2015). Albumin showed a protective effect on bevacizumab activity and stability in PLGA nanoparticles to prevent Ab inactivation and aggregation (Varshochian et al. 2013).

Requirements and challenges of Ab drug-loaded ocular delivery systems

To apply an Ab drug-based delivery system via intraocular route, safety evaluations (Hansel et al. 2010) and

identification of the physicochemical properties of the delivery system are recommended (Baranowski et al. 2014). Toxicity tests should be performed to evaluate the preclinical safety of Ab-based therapeutics as they are biotechnology-derived pharmaceuticals (ICH guideline S6, R1). European Medicines Agency (EMA) also issued the guideline on development, production, characterization, and specification for mAbs and related products. The general guidance documents supporting first-in-human trials with mAbs is listed in Table 3 (Muller and Brennan 2009). Tissue cross reactivity, in vivo preclinical safety, and immunogenicity (generation of antidrug antibodies, ADA) of Ab-based therapeutics are considered for regulations. Using delivery systems of Ab drugs, preclinical safety studies as well as release tests should be performed (Muller and Brennan 2009). The diffusivity of drugs from the delivery systems is one of critical factors for controlled release of drugs at targeted sites, and it is based on the evaluation of nanomaterials for their physicochemical properties such as hydrophobicity/hydrophilicity, size distribution, and morphology (Fig. 5).

Conclusion

Representative anti-VEGF drugs of aflibercept, bevacizumab, and ranibizumab are clinically used for the treatment of ocular diseases including wet AMD and myopic CNV. Ab drug-loaded ocular delivery systems of liposomes, hydrogel, and nanoparticles serve as promising tools for overcoming the limitations of Ab therapeutics (e.g., repeated dosing, poor bioavailability, and toxicity). Ocular platforms for modified release of Ab therapeutics are essential for the successful treatment of diseases based on effective delivery of drugs.

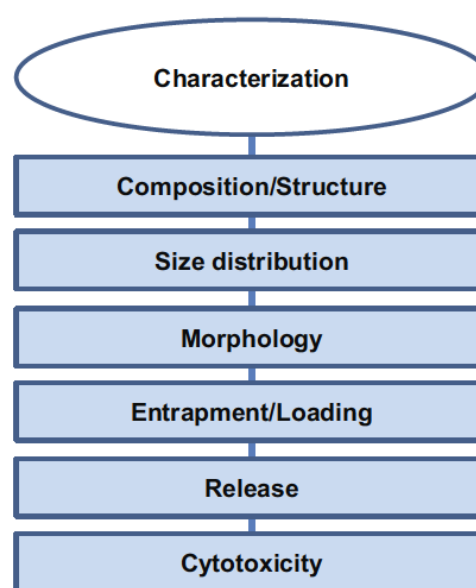


Fig. 5 Representative characterization criteria for nanopatform-based Ab therapeutics in ocular delivery

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain any studies with human and animal subjects performed by any of the authors.

Table 3 General guidelines for Ab therapeutics

Title	Authority	Year
Tissue cross-reactivity		
Points to consider in the manufacture and testing of monoclonal antibody products for human use	FDA/CBER	1997
Guideline on development, production, characterization and specification for monoclonal antibodies and related products	EMA/CHMP	2016
In vivo preclinical safety		
S6: Preclinical safety evaluation of biotechnology-derived pharmaceuticals	ICH	2011
S8: Immunotoxicity studies for human pharmaceuticals	ICH	2005
S9: Nonclinical evaluation for anticancer pharmaceuticals	ICH	2009
Immunogenicity (preclinical and clinical)		
Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins	EMA/CHMP	2008

FDA US Food and Drug Administration, *CBER* center for biologics evaluation and research, *EMA* European medicines agency, *CHMP* Committee for Medicinal Products for Human Use, *ICH* international conference on harmonization

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