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Ocular delivery of proteins and peptides: challenges and novel formulation approaches

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Abstract

The impact of proteins and peptides on the treatment of various conditions including ocular diseases over the past few decades has been advanced by substantial breakthroughs in structural biochemistry, genetic engineering, formulation and delivery approaches. Formulation and delivery of proteins and peptides, such as monoclonal antibodies, aptamers, recombinant proteins and peptides to ocular tissues poses significant challenges owing to their large size, poor permeation and susceptibility to degradation. A wide range of advanced drug delivery systems including polymeric controlled release systems, cell-based delivery and nanowafers are being exploited to overcome the challenges of frequent administration to ocular tissues. The next generation systems integrated with new delivery technologies are anticipated to generate improved efficacy and safety through the expansion of the therapeutic target space. This review will highlight recent advances in formulation and delivery strategies of protein and peptide based biopharmaceuticals. We will also describe the current state of proteins and peptides based ocular therapy and future therapeutic opportunities.

Graphical abstract

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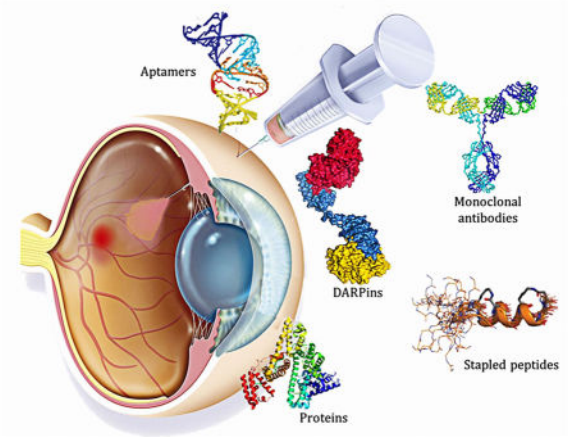

Keywords

Drug delivery; eye; barriers; targeting; AMD; biopharmaceuticals; controlled release; macromolecules; biologics

1. Introduction and current scenario of ophthalmology

In the past few decades, since the approval of a protein based biopharmaceutical in 1982 (Humulin; recombinant human insulin; Eli Lilly, Indianapolis), the approval rate of protein and peptide based biopharmaceuticals has grown significantly[1]. Of the top 10 pharmaceutical products by sales in 2014, a majority was biopharmaceuticals including recombinant therapeutic proteins, peptides, enzymes, monoclonal antibodies and antibody-drug conjugates. From 1982 to 2014, the total number of licensed biopharmaceutical products advanced from 13 to 246 in the United States (US) and European Union (EU; Brussels). The worldwide sales of biopharmaceutical drugs was estimated to be $289 billion in 2014 and are projected to grow to $445 billion by 2019[2]. Among these, the rapidly growing monoclonal antibody (mAb) therapeutics market itself has currently resulted in global sales of over US$50 billion [3]. Likewise with the inception of the anti-vascular endothelial growth factor (anti-VEGF) aptamer in 2004 (Macugen; Pegatanib sodium; OSI Pharmaceuticals, New York) and monoclonal antibody in 2006 (Lucentis; Ranibizumab; Genentech, California), the growth of ophthalmic protein and peptide based biopharmaceutical drug market has accelerated staggeringly. The global sales of biopharmaceutical drugs for ophthalmic indications had exceeded $8 billion in 2016 and is expected to reach $35.7 billion by 2025[4, 5]. A recent survey of ophthalmology market research revealed biologics and drug delivery systems to be the sectors that are anticipated to show strong growth in the next five years[6].

In addition to global sales and market, ophthalmology has garnered quite startling investments in terms of research funding in comparison to other disease areas indicating the urgent need for advanced therapeutic approaches for the treatment of chronic ocular diseases[7].

While over 900 new biopharmaceutical entities are in pipeline, targeting diseases across a wide range of therapeutic areas, the emergence of biosimilars is anticipated to represent the biggest shift in biologic approval landscape[8]. The U.S. patents for blockbuster Lucentis® will be expiring in 2019 and several biosimilar manufacturers are already targeting that molecule[9]. The current ophthalmic drug delivery technologies are tailored to non-targeted small molecules/drugs.

Biopharmaceuticals including proteins and peptides have shown great promise as novel therapeutics in the treatment of ocular diseases. These large molecules offer several advantages compared to small molecule drugs with respect to high potency, activity, low unspecific binding, less toxicity, minimization of drug-drug interaction, biological and chemical diversity [10, 11]. However, these macromolecules also face various challenges such as physical and chemical degradation, short in vivo half-lives, circulation, and distribution. Additionally, macromolecules lack efficient and specific delivery to the target sites. Besides these, clearance by the mononuclear phagocytes (MPS) of the reticuloendothelial system (RES), risk of immunogenic effect, high molecular weight (MW), structural complexity, and failure to permeate cell membranes further reduce their therapeutic efficacy [12]. For these reasons, there is a need to develop novel ophthalmic biopharmaceutical drugs and delivery systems, ideally targeting these macromolecules to biologically relevant ocular tissues.

2. Ocular diseases: current and future biologics based treatments

Millions of people worldwide suffer from a wide variety of ocular diseases. A majority of these pathologies lead to irreversible blindness thereby substantially reducing quality of life. The number of visually impaired people has escalated to 285 million worldwide currently. In the United states alone, one million people were legally blind (visual acuity of 20/200 vision or worse) while 3.2 million suffered from visual impairment and another 8.2 million had vision problems due to uncorrected refractive error in 2015. The number of these conditions are projected to double by 2050[13].

Last few decades have witnessed a considerable growth in the understanding of the pathogenesis and genetics of ocular diseases. Deciphering various compliment pathways, gene associations and pharmacological interventions for retinal diseases have led to substantial development of effective therapies[14]. The major ocular diseases that have significantly impacted vision worldwide include age-related macular degeneration (AMD), cataracts, diabetic retinopathy (DR), dry eye conditions and glaucoma. The treatment market for glaucoma had the largest market share in 2013 with product sales (both branded and generic) exceeding US$ 4.5 billion (£ 2.9 billion) in the United States, Europe and Japan combined. Age related diseases including cataracts, AMD and diabetic retinopathy are expected to become more common with aging populations in developed countries[6]. Table 1 lists FDA approved biopharmaceuticals for ocular indications.

2.1. Anti-VEGF agents

The ophthalmology market has grown tremendously over the last 20 years both financially and technologically. The biological milieu of human eye has attracted several proteins,

peptides and gene therapy based companies worldwide. Currently, the US Food and Drug Administration (FDA) has approved proteins and peptides based therapy for various ocular indications, involving anti-VEGF agents such as pegatanib (Macugen®), ranibizumab (Lucentis®), aflibercept (Eylea®) that serves as “VEGF trap”, and bevacizumab (Avastin®) which is used off label. Anti-VEGF therapies block the binding of VEGF signaling peptide to its receptors, neutralizing VEGF’s downstream effect of promoting growth of leaky blood vessels from the preexisting ones[20].

Pegatanib, a pegylated anti-VEGF aptamer binds to the major pathological VEGF-A isoform, VEGF165. VEGF165 is primarily responsible for mediating neovascularization in the eye. In contrast, ranibizumab and bevacizumab binds to all isoforms of VEGF-A. Ranibizumab (~48kDa), a monoclonal IgG1 antibody fragment has been reported to exhibit 17-fold higher binding capacity to VEGF receptors (VEGFRs) in comparison to full length bevacizumab (~149kDa). Aflibercept (~97kDa), unlike other VEGF inhibitors, is a recombinant fusion protein that acts as a dummy receptor for VEGF, thus effectively inhibiting the angiogenic response[21]. In addition, it’s ~200 fold higher affinity for VEGF in comparison to ranibizumab may be attributed to strong binding to VEGF-A, VEGF-B and PlGF (placental growth factor) and thereby influencing multiple pathways involved in cell proliferation, migration, extracellular matrix (ECM) degradation as well as pathological angiogenesis[22–24]. These protein and peptide based biopharmaceutical agents have remained relatively effective for the treatment of AMD and related ocular complications for the last few years. However, many patients do not respond to these treatments and some develop decreased responsiveness to the treatment itself. In fact repeated intravitreal injections requires skilled professional execution adding to the treatment cost and serious side effects including ocular pain, infection, or hemorrhage.

Recently, Vasotide, D(Cys-Leu-Pro-Arg-Cys) (a small cyclic retro-inverted peptidomimetic) developed by Sidman and his co-authors has demonstrated to uniquely block VEGF from binding to two different endothelial receptor molecules i.e. VEGF receptor-1 (VEGFR-1) and neuropilin-1(NRP-1) thus inhibiting retinal angiogenesis. While, VEGFR-1 is known to bind to VEGF ligands: VEGF-A, VEGF-B, and PlGF; NRP-1 modulates several VEGF isoforms including PIGF. Vasotide delivery through eye drops or intraperitoneal injection in three different animal models (a monkey model of human wet AMD, a mouse model of retinal angiomatous proliferation, and a mouse model of retinopathy of prematurity) have demonstrated effectiveness by inhibiting retinal angiogenesis. Such potential of Vasotide peptide in binding two important VEGFRs and at the same time blocking additional mechanisms holds promise for further translation into safer, less-invasive applications in retinal disorders (Fig. 4)[25]. Current anti-VEGF therapies are approved for neovascular (wet) AMD and diabetic macular edema (DME). However, these therapies are often used off-label for other ocular complications including corneal neovascularization and neovascular glaucoma. It has to be taken into consideration that such anti-VEGF therapies are not recommended by FDA to treat diseases such as central serous retinopathy and polypoidal choroidal vasculopathy where VEGF suppression is not the target. In addition, long-term or continuous blocking of VEGF may cause retinal atrophy and/or prevent normal vascular formation, which still remain unanswered.

2.2. Anti-TNF-α agents

Till date, a number of anti-tumor necrosis factor alpha (TNF-α) agents have been approved by FDA for the treatment of rheumatoid arthritis, ankylosing spondylitis, and psoriasis arthritis. Among these, Adalimumab (Humira®), a recombinant human IgG1 monoclonal antibody received FDA approval recently (July,2016) for the treatment of non-infectious intermediate, posterior, and panuveitis[26]. TNF-α plays an important role in the pathogenesis of inflammatory, edematous, neovascular, and neurodegenerative diseases [27]. In addition, there is increasing evidence of TNF-α involvement in the pathogenesis of experimental retinal neovascularization, proliferative vitreoretinopathy, and macular edema [28–30]. Adalimumab specifically binds to TNF-α and prevents its binding to TNF-α receptors (TNFR) thus blocking inflammatory responses. Fig. 5 depicts effects of anti-TNF-α agents (Adalimumab & infliximab) in treating retinal degeneration and ocular inflammation respectively. There are now enough evidences suggesting the important role of anti-TNF-α therapy in the management of ocular complications specially uveitis[31]. Although, increased risk of serious infections, malignancies and high cost are few drawbacks of such anti-TNF-α therapies [32], further development in delivery strategies for TNF-α blockers in treating diseases of the choroid, retina and macula may hold promise in improving vision and quality of life.

2.3. GLP-1 agonists

Exenatide (Byetta®/Bydureon®), Liraglutide (Victoza®/Saxenda®), albiglutide (Tanzeum®) and Dulaglutide (Trulicity®) are FDA approved glucagon-like peptide-1 (GLP-1) agonists indicated for the treatment of diabetes mellitus type 2. GLP-1 agonists bind to the glucagon-like peptide 1 receptor (GLP1R) to activate the adenylyl cyclase pathway resulting in increased insulin synthesis and release. GLP1R is highly expressed in pancreatic beta cells and the brain. Retina, being an ontogenetically brain-derived tissue is anticipated to express GLP1R [35]. Recently, Hernandez and co-authors reported abundant expression of GLP1R in human and nonketotic diabetes mice retinas. Retinal degeneration can be treated with systemic administration of liraglutide which was evident from significant reduction in extracellular glutamate levels and increase in prosurvival signaling pathways (Fig. 6). In addition, similar neuroprotective effect was demonstrated after topical administration of native GLP-1 and other GLP-1R agonists without any reduction in blood glucose levels. Such GLP1R expression and activation may open up new approaches for preventing or arresting retinal neurodegeneration with GLP-1 agonists in early stages of diabetic retinopathy [36].

2.4. Next generation protein and peptide based therapies

Significant developments in protein and peptide based therapies have recently led a number of biologics to enter into clinical trials. For instance, Abicipar pegol (previously AGN-150998 or MP0112, Molecular partners and Allergan) is a genetically engineered mimetic antibody derived from designed ankyrin repeat protein (DARPin®) family. Abicipar is a long-acting mono-DARPin® that binds to all VEGF-A isoforms with high specificity and affinity, thus adding to its good molecular stability, tissue penetration and ease of manufacturing [37]. Abicipar has successfully completed Phase I/IIb clinical trials in wet

AMD and DME and is currently recruiting participants for Phase 3 studies (NCT02462928) [38]. Brolucizumab (Alcon Laboratories Inc.), a humanized single-chain variable fragment that binds to all isoforms of VEGF-A with high affinity has completed Phase II clinical trials in wet AMD.

Pegpleranib (Fovista®, Ophthotec) is an anti-platelet-derived growth factor (anti-PDGF) agent that binds to PDGF-BB and prevents PDGF binding to PDGF-β receptors on pericytes, leading to their death via interruption of cell survival signals [39]. Fovista® is currently undergoing Phase 3 clinical trials for the treatment of wet AMD (NCT01940887). Rinucumab (Regeneron), a monoclonal antibody, binds to the PDGF-β receptor, thus prevents the action of PDGF. Rinucumab is in Phase II clinical trial and is being employed in combination with aflibercept in a co-formulated single injection for wet AMD.

Nesvacumab (Regeneron), a monoclonal antibody against angiopoietin-2 (ANG-2) is currently in a Phase II clinical trial and is indicated in combination with aflibercept in a co-formulated single injection for wet AMD. RG7716 (Hoffmann-La Roche), a bispecific antibody that binds both VEGF A and ANG 2 is in Phase II study. It is currently indicated as combination therapy along with Lucentis® for patients with wet AMD (NCT02484690).

Zimura® (Ophthotec), a chemically synthesized anti-C5 aptamer that inhibits complement factor 5 (C5), which is the fifth component participating in cellular inflammatory process. It is in Phase II study for geographic atrophy secondary to dry AMD (NCT02686658), and a combination therapy with Lucentis® has recently completed Phase IIa clinical trials for wet AMD. HI-con1 (Iconic Pharmaceuticals) is a human fusion immunoprotein consisting of two human factor VII as the targeting domains fused to IgG Fc as an effector domain. This chimeric protein binds to tissue factor (TF) with the factor VII component, while the IgG component triggers destruction of the neovascular lesion. It is currently undergoing Phase II study as a monotherapy and/or in combination with Lucentis®. Opt-302 (Opthea) is another fusion protein that binds VEGF-C and VEGF-D, blocking their interaction with VEGFR-2 and VEGFR-3. Opt-302 is also in a Phase I/IIA trial for wet AMD. Apart from these, POT-4 (Alcon, Phase I), Eculizumab (Alexion, Phase II), LFG316 (Novartis, Phase II), FCFD4514S (Genentech, Phase II), Sonepcizumab (Lpath, Phase II), Glatiramer acetate (Teva, Phase II/III), RN6G (Pfizer, Phase II), Daclizumab (Hoffman-La Roche, Phase II) and Infliximab (Janssen, Phase II) are under various stages in clinical trials. Several other protein and peptide based therapeutics are under development [40]. Table 2. lists some of the proteins and peptides currently in clinical trials.

3. Proteins and peptides: challenges in ocular delivery

Proteins and peptides, a class of biopharmaceuticals poses significant challenges owing to their large size, poor permeation and susceptibility to degradation. The intrinsic properties associated with the complex macromolecular nature of proteins and peptides is often required for achieving high biological activity. However, such structural complexity also renders them as one of the most challenging class of therapeutics to be formulated and delivered. Low stability and short half-lives of peptides and especially protein drugs at

physiological pH and temperature or during storage leads to loss of activity, thus putting significant burden on formulation technologies.

3.1. Adverse physicochemical properties of proteins and peptides

3.1.1. Hydrophilicity—Most of the therapeutic proteins and peptides are highly hydrophilic (log P<0) which hinder their permeability across biological membranes. Bioavailability of proteins and peptides depends on their ability to cross these membranes. Poor membrane permeation of macromolecules often embodies added challenge in development of protein and peptide based drug formulations to intracellular target sites. The lipophilic nature of biological membranes restrict these macromolecules from spontaneously entering cells. The absorption of these macromolecules is not governed by simple diffusion or passive absorption. Rather active transport which involves binding to specific receptor, pinocytocis or endocytosis are the major mechanisms responsible for absorption [42, 43]. Permeation of hydrophilic molecules is hindered by the tight junctions present in the cornea and the lipophilic nature of the corneal epithelium [44, 45] whereas hydrophobic molecules permeate corneal epithelium easily. Additionally, the collagen fibers present in the hydrophilic stroma may impede penetration of hydrophobic drugs to some extent. Under certain circumstances, small peptides or even small particles are taken from the extracellular space into cells by an active transport mechanism known as receptor-mediated endocytosis [60]. One of the major disadvantages of proteins and peptides entering into the cell via endocytic pathway is their entrapment into the endosomes and eventually in lysosomes, where majority of the degradation processes undergoes by the action of lysosomal enzymes. This leads to only a small fraction of unaffected proteins/peptides appearing in the cytoplasm. So far, multiple and partially successful attempts have been made to deliver protein and peptide based biopharmaceuticals directly into the cell cytoplasm bypassing the endocytic pathway. Mechanical delivery methods like microinjection and electroporation have been used for decades for cell cytoplasm delivery, but are low-throughput and invasive and require specialized equipment to physically puncture membranes. The delivery of biologics via most favored “oral route” is highly challenging due to GI mucosa and degradative acidic environment. A large fraction of approved and investigational protein and peptide molecules are administered via parenteral routes (IV, IM or SC), intravitreal and sub conjunctival injections. However, non-targeted delivery of protein and peptide based formulations may lead to distribution into normal tissues requiring large quantities of drug administration, which is often not economical and sometimes complicated owing to non-specific toxicity.

3.1.2. Large molecular weight—Another major challenge for the delivery of protein and peptide based drugs is their high molecular weight and poor membrane permeability across ocular tissues and barriers. Such challenges have promoted highly invasive intravitreal injection as the primary mode administration for protein and peptide based drugs. The molecular weights of peptides and proteins are generally > 1000 Da with large hydrogen bonding donor/ acceptor groups [46]. Such large size of macromolecules limits diffusion and renders patient compliant topical treatment highly inefficient. The cornea, sclera and retina have tight junctions that significantly limits diffusion of hydrophilic large molecules [47, 48]. The tight junctional space of conjunctival epithelium is generally wider than cornea, but

still insufficient for the penetration of these large molecules [5, 49]. The human retina limits the diffusion of molecules greater than 76 kDa due to the inner and outer plexform layers. Macromolecules greater than 150 kDa fail to reach the inner retina [47]. Additionally, choriocapillaries may wash out the molecules that traverse through choroid thus reducing therapeutic concentrations. The ocular anatomy and tissue barriers are shown in Fig. 7.

3.1.3. Metabolic instability—Proteins and peptides also suffer from a number of physical, chemical and biological instability issues due to their complex secondary, tertiary and quaternary structures. Various physical degradation pathways are involved in the instability of proteins and peptides including denaturation, adsorption, aggregation and precipitation. Moreover, conformational transformation of proteins to inactive forms occur due to pH, temperature, high salt concentration; dissociation of subunit proteins; complexation of enzymes and cofactors; non-covalent complexation with ions, proteolytic degradation under the influence of esterases and proteases; chemical modifications by different compounds (for instance oxidation of SH-groups in sulfhydryl containing enzymes and Fe (II) atoms in heme containing proteins; thiol-disulfide exchange and destruction of labile side-chains of tryptophan and methionine) may also lead to inactivation of various biologically active protein and peptide based drugs in ocular tissues [11].

In the body, the chemical degradation pathways of peptides and proteins include deamidation, oxidation and reduction, proteolysis, disulfide exchange and β-elimination [63]. Any alteration in “active” confirmation may lead to loss of activity and irreversible aggregation of proteins. Vulnerability towards enzymatic degradation under in vivo condition results into shorter half-lives even with parenteral administration. Inside the vitreous humor the half-life of large molecule tends to be in the range of days to weeks [64]. Such short half-lives of proteins require frequent parenteral administrations to maintain therapeutic levels. Frequent parenteral administrations are not patient compliant and/or well tolerated and are often associated with complications including cataract, retinal hemorrhage and detachment [65]. For instance, the average apparent plasma half-life of pegaptanib is 10 days after 3 mg dose whereas ranibizumab remains for 2.88 days in rabbit. Half-life of bevacizumab is 4.32 days with maximum concentration 162 μg/ml in vitreous cavity [66]. In AMD, the vitreous elimination of ranizumab is just 9 days and intrinsic systemic elimination half-life is 2 hours followed by multiple intravitreal injection dose of 0.3–2.0 mg/eye biweekly or monthly [17].

3.2. Challenges in designing protein and peptide based ocular formulations

The formulation of protein and peptide based biotherapeutics poses unique challenges that are not often experienced with small molecules. Overcoming the instability of protein and peptide based agents due to structural properties and environmental factors is one of the key challenges in the development of formulations. Several agents have been incorporated including small sugars (e.g. trehalose) and polysaccharides (e.g. dextrans) to enhance the stability of protein and peptide based biopharmaceuticals [67, 68]. Pluronics and non-ionic surfactants such as polysorbates at low concentrations are widely applied to decrease protein and peptide aggregation [69].

Another major drawback of biopharmaceutical drug formulations is high and variable viscosity. For topical ophthalmic formulations, corneal contact time is longer with increase in viscosity of formulations up to 20 centipoise (cP) [70]. However, a further increase in viscosity leads to reflex tearing and blinking in order to regain the original viscosity of the lacrimal fluid (1.05–5.97 cP). With a rise in clinical application of monoclonal antibodies, the need for high protein doses (concentrated formulations) is often crucial. The FDA does not permit the intravitreal injection of large volumes of drug formulations in patients with ocular diseases. Such requirements render formulation of protein and peptide based biopharmaceuticals very difficult as solutions with high protein content are exceedingly viscous. High viscosity of protein and peptide based biopharmaceuticals also largely affects the syringeability (time required to complete the injection) as well as the force required to deliver the solution with appropriate needles (18 mm in length, 27–30G) [10]. Thus, approaches to achieve lower viscosity formulations with hydrophobic/inorganic salts or lysine and arginine may be useful.

It is also important for protein and peptide based biopharmaceutical formulations to have the same pH as the lacrimal fluid to achieve maximum activity. However, proteins and peptides are often not stable at physiological pH leading to their folding and aggregation. Additionally, the buffer capacity of such formulations is of equal importance for proper preservation. Although, the buffering action of the tears is capable of neutralizing the effect of topically applied biopharmaceutical formulations[71], intraocular hyperosmotic solutions have been reported to elicit transient desiccation of the anterior chamber tissues while hypotonic solutions may cause edema leading to corneal clouding[72]. For this reason, pH of such formulations are compromised and maintained by buffers to achieve maximum activity and maintain stability[73]. The effect of buffers on tonicity should also be taken into account considering the permissible limits of osmolarity for ophthalmic formulations (171–1711 mOsm/kg). Although many of these agents utilized for maintaining the stability and activity of such protein and peptide based biopharmaceutical formulations have been proven to be effective, their use requires careful consideration in terms of local toxicity and potential immunogenicity.

A better understanding of the viscosities of biological solutions, characteristics of nascent proteins and peptides, dynamics and behavior of protein and peptide based topical and injectable formulations is crucial. Towards this goal, utilization of chemical chaperones to inhibit protein misfolding as well as reactivate non-native protein structures[74, 75]; co-administration of recombinant human hyaluronidase with drug to degrade hyaluronic acid (a key structural component of tissues) to facilitate protein and peptide delivery may prove to be useful in addressing the issues poised by formulation challenges[76].

3.2.1. Recombinant human hyaluronidase: penetration enhancer—Hyaluronan (HA), a unique polyanionic and protein-free polysaccharide is highly expressed in the vitreous humor and is primarily responsible for increasing viscosity, expanding volume, and providing structural support to the vitreous body. However, the high viscosity of HA allows it to act as a molecular sieve, thus preventing the penetration of most biopharmaceutical formulations. The property of hyaluronidase (Hyal) to catalyze the degradation of HA have

been exploited for decades to increase the penetration of biopharmaceutical drugs across ocular tissue barriers [77, 78].

Human Hyal-1(hHyal-1) is one of the five homologous hyaluronidases encoded in the human genome (Fig. 8A). It is highly expressed in most tissues and cleaves HA substrates of all sizes in a size-independent manner to tetrasaccharides. The amino acid residues present in the N-terminal of hHyal-1 exhibit 31% sequence identity with bee venom hyaluronidase (bvHyal), whose structure has been shown in complex with a HA tetrasaccharide (Figs. 8B and C). In addition, the EGF domains present in Hyal-1 are thought to mediate protein-protein interactions often associated with regulation of growth and development [79]. However, with the development of recombinant human hyaluronidase, some of the significant limitations including immune reactivity with bovine hyaluronidase and lack of catalytic activity at neutral pH with hHyal-1 have been addressed. Such developments have led to approval of HYQVIA (Baxter International Inc.), containing immune globulin infusion 10% (Human) with recombinant human hyaluronidase for adult patients with primary immunodeficiency. PEGylated recombinant human hyaluronidase are currently undergoing Phase III clinical trials in combination with paclitaxel and gemcitabine for treating pancreatic ductal adenocarcinoma (NCT02715804). rHuPH20, another purified form of the recombinant human hyaluronidase has shown promise in elevating dexamethasone levels in ocular tissues (choroid and retina) and the serum [80]. It is currently undergoing Phase I clinical trial for multiple myeloma (NCT02519452) [81]. Although inhibiting a key stromal component such as HA might cause some immunogenic reactions in the body, such potential of recombinant human hyaluronidase in facilitating drug delivery holds promise in the development of protein and peptide based ocular formulations.

3.2.2. Chemical chaperones: protein aggregation inhibitor—Protein aggregation has remained as one of the primary concerns in the formulation of protein and peptide based biopharmaceuticals for ocular diseases. Previously, several small molecules have been identified for modifying or inhibiting protein aggregation. A novel strategy developed by Sanders et al. utilizes chemical chaperones to inhibit protein misfolding by (kinetic) stabilization and/or inhibit the self-assembly of aggregation-prone sequences of the native protein structures (Fig. 9A)[75].

In addition, there is growing evidence that several ocular diseases including cataract compromises the folding of the endogenous proteome by sequestering chaperones and chaperonins leading to intra-cytoplasmic aggregation of proteins involved in critical cellular processes [82]. It has been reported that crystallins, constitute 90% of the total proteins in mature lens and undergoes covalent modifications and/or polymerization (Fig. 9B) causing destabilization and aggregation of lens proteins. α-Crystallin, a major chaperone system of mature lens cells recognizes and sequesters misfolded/unfolded conformers, reducing the accessibility of aggregation prone species [83, 84]. Therefore, the application of chaperones provides exciting opportunities for modulating protein aggregation in biopharmaceutical formulations as well as lowering protein aggregate-induced toxicity. Glycerol, 4-Phenylbutyric Acid Sodium Salt (PBA), Tauroursodeoxcholic acid (TUDCA) and trimethylamine-N-oxide (TMAO) have gained wide application as chemical chaperones.

Apart from these small molecules, endogenous molecular chaperones (e.g. heat shock proteins, Hsp) and pharmacoperones (e.g. nicotine) are being extensively exploited to promote folding of specific proteins [85].

4. Types of protein and peptide modifications

Several intraocular delivery techniques including intrastromal, intracameral, suprachoroidal and intravitreal injections have been explored as possible ways for the delivery of biopharmaceuticals across ocular barriers. Intravitreal injection is currently the most commonly used method for delivering proteins and peptides to the back of the eye. Regardless of the type of injection, most biopharmaceuticals are rapidly cleared from the ocular tissues through posterior transretinal and anterior aqueous humor elimination pathways [87]. Consequently frequent and repeated injections are required which impose a significant treatment burden on the patient, vision care providers, and a cumulative risk of adverse effects from each subsequent injection [88, 89]. Various strategies have been developed and summarized to overcome and address such challenges of proteins and peptides delivery in the next section:

4.1. Chemical modifications

Chemical modification with hydrophilic polymers is a useful strategy to improve the hydrodynamic diameter of the therapeutic constructs which can reduce clearance and promote circulating half-life to an attractive range. PEGylation is one such strategy that involves covalent attachment of a FDA approved polymer, polyethylene glycol (PEG) to a primary amino (-NH_2) or sulfhydryl (-SH) groups of proteins or peptides. PEG chains of molecular weight ranging from 5–40 kDa have shown to improve biological activity of therapeutic proteins or peptides and reduce immune responses to a larger extent. Such developments have led to approval of several pegylated drugs in the market [90, 91]. Alternatives to PEG, the negative charge of sialic acid as well as the glycosaminoglycan HA and hydroxyl ethyl starch also holds potential in prolonging half-lives of proteins and peptides and are currently under clinical investigation[92].

4.2. Genetic engineering based modifications

The neonatal Fc receptor (FcRn) is a unique protein encoded by the Fc fragment of IgG receptor and transporter (FCGRT) gene in human. It is similar in structure to the major histocompatibility complex (MHC) class of molecules. FcRn's exceptional ability to protect IgG and albumin from catabolism (Fig. 10) has guided development of novel genetic fusion based biopharmaceuticals. With higher expression of FcRn in various ocular tissues including corneal epithelium and endothelium, lens epithelium, retinal blood vessel, conjunctiva lymphatic vessel, nonpigmented ciliary epithelium, ciliary blood vessel, iris blood vessel, and optic nerve, approaches to exploit FcRn pathway can be extended to improve circulating time and half-lives of various therapeutic proteins and peptides for ocular delivery [93, 94]. So far only a few approaches to modulate IgG- and albumin-FcRn interactions have been reported. They involve mutations of Fc-domain amino acid residues in the proximity to the FcRn binding site and engineering the IgG– and albumin- FcRn interactions to increase antibody/albumin half-lives [95]. Zalevsky and co-authors

demonstrated mutations of two amino acid residues in the human IgG1 VEGF antibody bevacizumab resulted in a ~11-fold improvement in the affinity for human FcRn at pH 6 (Fig. 11) [96]. In addition, bispecific antibodies (bsAbs) co-targeting the PDGF and VEGF pathways to enhance the treatment of AMD have led to tailoring of antibody-like proteins for specific needs [97]. However, the immunogenicity, processing and manufacturability of these bsAbs continue to be a major hurdle for clinical approval. Although, a number of fusion proteins have been recently approved by FDA for various indications and some are undergoing clinical trials, none have been approved for ocular indications.

Newer insights on protein and peptide modifications based on medicinal chemistry and structure-activity studies including use of hydroxyl-PEG as an alternative to widely used methoxy-PEG, supramolecular PEGylation of macromolecules for higher binding affinity [98], reversible pegylation to mitigate reduced potency and use of amphiphilic poly(2-oxazoline) polymers which provides better control of the molecular definition of biopharmaceuticals may offer improvements in the pharmacokinetics and potency of protein and peptide based biopharmaceuticals.

5. Routes of protein and peptide delivery to ocular tissues

Challenges to ocular delivery of biopharmaceuticals are noteworthy and considerable opportunities remain to be optimized for delivery approaches, formulation and processing conditions for each peptide and protein based therapeutics.

5.1. Systemic delivery

Oral administration and parenteral injections are typical methods employed to achieve systemic delivery. However, attempts to deliver large hydrophilic protein and peptide based biopharmaceuticals for ocular indications have seen limited success. The miniature size of the eye and presence of ocular barriers prevent ample drug partitioning into the eye. Furthermore, dilution effect of the systemic blood volume, first-pass metabolism by the liver and clearance by kidney require larger drug doses which can result in high costs, systemic side-effects and possible toxicity.

The integrity of ocular barriers seems to play a major role in the penetration of biopharmaceuticals. A study in a clinical set-up showed an increase in visual acuity by 14 letters after treatment with 3 doses of systemic bevacizumab (5mg/kg) in patients with classic choroidal neovascularization (CNV) probably facilitated by the compromised RPE layer [99]. Rohrer and co-authors also reported reduced CNV size and preserved retinal function after intravenous administration of fusion protein CR2-fH (where CR2 is complement receptor 2 and fH is factor H) indicating CR2-fH accesses the site of CNV by way of the impaired BRB. CR2-fH plays a critical role in regulating the inflammatory responses by inhibiting complement activation products in AMD [100, 101]. Although, no serious ocular or systemic side effects were observed in both the cases, high concentration of injected drug or fusion proteins should be taken into consideration. Such shortcomings preclude systemic administration of protein and peptide based biopharmaceuticals for ocular delivery expensive and rare.

5.2. Extraocular delivery

5.2.1. Topical delivery—Topical application of ophthalmic drops has been the method of choice for administering pharmaceutical agents for the treatment of diseases perturbing the ocular surface and/or the anterior segment including dry eye syndrome, conjunctivitis and keratitis. This route has been extensively utilized clinically for the treatment of diseases affecting cornea, conjunctiva, sclera, iris, ciliary body and aqueous humor. However, the limited lacrimal capacity and constant tear drainage from precorneal area leads to wash out of a majority of eye drop within few seconds. Additionally, only a few experimental studies have demonstrated their efficacy for posterior segment diseases. The properties of corneal barriers allow significant passage of moderately lipophilic small molecules, whereas highly hydrophilic large molecular weight biopharmaceuticals undergo restricted permeation generating insufficient concentrations for therapy. Nomoto and co-authors demonstrated the incompetence of topical bevacizumab to reach therapeutic concentrations in the iris, choroid, retina and vitreous of rabbits even after aggressive dosing of 1.25mg/0.05mL six times daily for a week [102]. In another study, topical administration of bevacizumab (10mg/kg, 3 times for 7 days) in mice did not generate any appreciable concentrations into the healthy corneal stroma [103]. In a recent study, Moisseiev and group also failed to generate detectable drug levels in both aqueous and vitreous samples of human eyes after topical administration of bevacizumab (25mg/mL, four drops with 10 minutes interval) [19]. In contrast, Hernandez and coworkers provided the first evidence that somatostatin (SST) eye drops reached the retina not through the cornea but by the trans-scleral route. Such topical administration of SST prevented retinal neurodegeneration in streptozotocin induced diabetes mellitus (STZ-DM) rats and opened up new preventive pharmacological strategy targeted to early stages of DR. [104].

5.2.2. Periocular delivery

5.2.2.1. Subconjunctival delivery: Periocular delivery is frequently achieved through an injection into the subconjunctival area i.e. space underneath the conjunctiva. An injection rooted into the bulbar conjunctiva and superficial to the sclera may provide a way to directly deliver therapeutics into the subconjunctival space. Subconjunctival routes can be used for sustained delivery since a depot can be formed in the space that can expand and accommodate up to 500 μL volume. However, drugs injected into the subconjunctival space are often rapidly cleared via conjunctival blood and lymphatic flow. In addition, pore diameter and intracellular spaces of scleral fiber matrix regulate drug permeation to a large extent. Longer in vivo $t_{1/2}$ in the iris/ciliary body and retina/ choroid after subconjunctival injection of bevacizumab relative to intravitreal injection may possibly be attributed to binding with negatively charged scleral proteoglycans [102]. In another in vivo study, high bevacizumab concentration was detected in the whole cornea post 24 hours subcutaneous injection which remained almost unchanged in all layers of stroma over the next 14 days [103]. Various drug delivery technologies including microparticles/nanoparticles may be combined with physical techniques such as ultrasound and iontophoresis to achieve therapeutic concentrations of protein and peptide based biopharmaceuticals following periocular administration [105, 106].

5.2.2.2. Sub-tenon delivery: Sub-tenon route is widely utilized for administering anesthetics during ocular surgery. It involves the injection of drug into a fibrous membrane, called tenon's capsule which along with the sclera binds the sub-tenon space. Although upto 4 mL of drug formulation could be injected through this route, administration complications including pain, chemosis, subconjunctival hemorrhage, retrobulbar and/or orbital hemorrhage, optic nerve damage, retinal ischemia, orbital swelling and rectus muscle dysfunction limit its use for the delivery of protein and peptide based biopharmaceuticals [107, 108]. In patients with clinically significant macular edema, sub-tenon's injection of bevacizumab (2.5 mg in 0.1 mm volume) resulted in significant short-term visual improvement in eyes [109]. Thus, sub-tenon's injection may serve as an alternative to intravitreal injection for ocular delivery of biopharmaceuticals.

5.3. Intraocular delivery

Intraocular delivery techniques involve direct delivery of therapeutic agents to the target site thus reducing the distance traversed by the drug to generate higher local drug concentrations, reduced off-target effects and bypassing various ocular barriers to improve ocular drug bioavailability.

5.3.1. Intrastromal delivery—Intrastromal administration entails direct drug delivery into the corneal stroma to overcome the corneal epithelial barrier along with tear fluid drainage. The densely packed collagen fibrils and proteoglycans hinder the diffusion of proteins and peptides inside the corneal stromal structure allowing it to serve as a reservoir for large hydrophilic biopharmaceuticals. Hashemian and co-authors reported intrastromal injection of bevacizumab (2.5 mg/1 mL) using a hypodermic needle led to regression of corneal stromal vascularization in a patient [110]. Recently, in vivo studies by Kim and group have demonstrated corneal vascular regression after intrastromal administration of bevacizumab (4.4 μg) with microneedles (MNs) [111]. These studies further confirm intrastromal delivery as an attractive modality for delivering biopharmaceuticals directly into the cornea.

5.3.2. Intracameral delivery—Intracameral delivery is intended to place the drug solution directly into the anterior segment of the eye. Although, intracameral injection has been extensively explored to improve delivery of biopharmaceuticals to both the anterior as well as posterior segments of the eye, it has not been possible to achieve therapeutic drug concentrations in the posterior segment of the eye following intracameral administration. However, intracameral administration of antibiotic prophylaxis for cataract surgery to prevent endopthalmitis [112, 113] and antifungal agents for deep corneal infections such as fungal keratitis [114] is widely used to deliver drugs to the anterior segment of the eye. Additionally, a combination of intrastromal and intracameral injections has recently shown to be effective in reducing fungal mass not only in the anterior segment but also in the corneal stroma where fungal invasion may lead to corneal perforation [115].

Several in vitro and in vivo studies have demonstrated the effectiveness of intracameral bevacizumab in treating neovascularization with no effects on corneal endothelial cells or thickness [116–118]. Patients with neovascular glaucoma and iris rubeosis have also

responded well to the intracameral bevacizumab therapy and did not show any morphological changes of corneal endothelial cells [119–121]. Intracameral injection of bevacizumab loaded polymeric delivery systems may sustain drug release into the anterior segment [122]. However, repeated injections to maintain therapeutic concentrations over prolonged time period and sparse degradation of polymers may obstruct the aqueous flow, thereby elevating intraocular pressure and risk of ocular infections.

5.3.3. Intravitreal delivery—Intravitreal injection is the main modality for delivering biopharmaceuticals to the posterior segment of the eye to date. It is an invasive procedure that involves injection of a drug solution and/or suspension into the vitreous cavity in the center of the eye after penetrating through all layers of the ocular globe. The vitreous cavity can generally accommodate a volume of 20–100μL drug solution/suspension without resentfully altering the visual axis. However, various common complications including edopthalmitis, intraocular inflammation, retinal detachment, intraocular pressure elevation or glaucoma, ocular hemorrhage, floaters and cataract after intravitreal injections may lead to permanent vision loss if untreated.

Currently most of the biopharmaceuticals including pegatanib sodium, ranibizumab, aflibercept and bavcizumab indicated for neovascular or wet AMD are given as intravitreal injections. A comparative pharmacokinetic analysis revealed concentration (Cmax) of bevacizumab in retina/choroid after an intravitreal injection (1.25 mg/0.05 mL) to be ~317-fold higher than a subconjunctival injection at 1 week in rabbits [102]. Intravitreal injection of Avastin® generated significant bevacizumab concentrations in the retina, the retinal pigment epithelium, the choroid and particularly the photoreceptor outer segments in cynomolgus monkeys [123]. Although, biopharmaceutical drugs due to their large molecular weight tend to prevent immediate elimination from the vitreous unlike small molecules, their vitreous half-lives of just few days to weeks may not be sufficient to achieve long-term therapeutic effect. Therefore, novel delivery methods and/or long-term controlled release formulations for protein and peptide based biopharmaceuticals are warranted in order to significantly reduce complications caused by repeated injections.

5.3.4. Suprachoroidal delivery—It is often overlooked that the tissue site of action for most of the biopharmaceuticals is not the vitreous but the choroid and retina. Therefore, delivering drug directly in the target tissues (i.e., choroid and retina) may provide more effective therapy to chorioretinal diseases. Suprachoroidal injections, that involve the placement of a drug in the suprachoridal space (SCS), a conceivable space between the sclera and the choroid holds potential in achieving higher drug levels in target tissues. SCS can expand to accommodate a drug suspension or solution up to 1 mL [124]. Previously, SCS was accessed surgically with a scleral incision and insertion of a long cannula or hypodermic needle through the SCS. Such surgical interventions often lead to SCS collapse resulting from dislocation of the chorioretina and elevated hydrostatic pressure in the eye. Recent advancements in suprachoroidal delivery using MNs, has enabled higher local drug concentrations in the choroid with minimal side effects and least obstruction of the visual acuity. Nonetheless, high blood flow in choriocapillaries render the half-lives of small molecules and biopharmaceuticals in SCS in the order of few minutes to hours. In fact,

sustained delivery systems (20 nm – 10 μm) are retained in the SCS for longer periods indicating the suitability of SCS injections [125, 126].

Several studies have demonstrated the effectiveness of suprachoroidal injections for localized delivery of therapeutics to the choroid-retina region [127]. Although, microcannula suprachoroidal injections have shown not to sustain the release of bevacizumab as superiorly as intravitreal injections post one week [128], MNs have demonstrated the potential in delivering bevacizumab (100 μl) to the SCS without any serious adverse effects as noted in Phase I clinical trials [129, 130]. Fig. 12 depicts current and emerging routes for protein and peptide delivery to ocular tissues.

Nonetheless, inflammation which is a common side effect of ocular diseases including neovascularization significantly affects the integrity of corneal epithelium, choroid and the RPE layer. Such incompetent barrier function allows protein and peptide based biopharmaceuticals, that have limited access to the intact eye to gain significant access through the compromised barriers of inflamed eyes. Several studies to date have shown the effectiveness of systemic, intravitreal and SCS delivery in compromised tissues and confines compelling implications for other biological approaches in the treatment of ocular diseases. Some characteristics of various routes of administration for ocular drug delivery are provided in Table 4.

6. Novel formulation approaches for ocular delivery of proteins and peptides

6.1. Biodegradable polymeric micro particles/microspheres

Micro particles or microspheres are generally employed for long-term ocular delivery (1 week or longer) of proteins, peptides and small molecules. The biocompatible polymers constituting the microspheres generate monomers and other nontoxic byproducts upon degradation that are safely cleared out from the eye and eventually from the systemic circulation. Poly(lactic-co-glycolic acid) (PLGA) are the most commonly used polymers with high encapsulation efficiency, sustained release, biocompatibility and ability to degrade into toxicologically acceptable products that are cleared out of ocular tissues [131]. Other potentially constructive materials include polyanhydrides [132] and cyclodextrins [133]. The protein or peptide release rate is closely related to structural properties of microspheres i.e. degradation rate of polymer and/or diffusion of the protein or peptide from the microsphere. In addition, the diffusion rates also depends on the molecular mass of the polymer, protein and peptide, molar ratio of lactic/glycolic acid, entrapment efficiency, surface charge, size and porosity. The shape of the particles also influences their behavior to a great extent [134]. Transcleral delivery of PLGA microspheres provided pegatinib sodium over a period of up to 20 days at the scleral surface [135]. Similarly, intravitreal injection of pegaptanib microspheres sustained release of pegaptanib over several weeks [136]. Gavini and co-workers reported appreciable vancomycin concentrations (0.81 mg/ml) from PLGA micropsheres in the rabbit aqueous humor 180 minutes after topical administration [137]. Such microspheres can be mixed with a fluid carrier in order to achieve better control over the release and pharmacokinetic profiles of the protein and/or peptide based

biopharmaceuticals [138]. PLGA microspheres suspended in poly(N-isopropylacrylamide) injectable thermo-responsive hydrogel have shown to sustain the release of ranibizumab (0.153 μg/day) and aflibercept (0.065 μg/day) for 196 days after initial burst release of 22.2 ± 2.2 and 13.1 ± 0.5 μg respectively [139]. The encapsulation process for proteins and peptides is more challenging because these macromolecules often lose their structure and biological activity upon interaction with polymeric materials and biological fluids [140]. For example, formation of covalent dimer by darbepoetin alfa (Aranesp®; Amgen) in a microsphere [141] and acylation of octreotide (Sandostatin® LAR depot, Novartis Pharmaceuticals) in PLGA microspheres [142]. To overcome the challenges of protein/peptide degradation and burst release, various strategies of hydrophobic ion-pairing (HIP) complexation, utilization of biocaompatible block-copolymers and on-demand drug release including pH, thermo, enzyme, light, ultrasound and multi responsive systems have been developed for ocular delivery [143]. Our laboratory has previously demonstrated the potential of such strategies including HIP complexation and block polymers in gel based formulations in sustaining release (~3 months) and minimizing acylation (<7%) of octreotide from microparticles [142, 144]. Fig. 13 shows an example of on-demand micro particle based drug release system. Although several microsphere formulations for ophthalmic indications have reached early stages of clinical trials, but none have yet been approved for commercialization[145]. It is very challenging to achieve long-term release and constant therapeutic levels of biopharmaceuticals for more than a week to months in ocular tissues using polymeric microspheres.

6.2. Biodegradable polymeric nanoparticles/nanospheres

Nanoparticles are generally composed of biodegradable polymers and lipids and include liposomes, dendrimers, micelles and nanowafers that are actively used as carriers for targeted delivery of proteins, peptides and small molecules. Likewise micro particles, drug release from nanoparticles is dependent on the rate of degradation of polymers, molecular mass and other physicochemical factors. Nanoparticles can be administered via various routes including topical, periocular, suprachoroidal and intravitreal. However, intravitreal injection often leads to clouding of the vitreous due to scattering of light by polymeric particles. While micro particles tend to sink to the lower part of the vitreal cavity attributed to their higher molecular mass, nanoparticles are more susceptible to cause clouding in the vitreous. In addition, possible bioactivity loss and low stability of biopharmaceuticals due to interactions with nanoparticle matrix and extensive nanoencapsulation methods may further complicate delivery of proteins and peptides based nano formulations.

While there are several examples of nanoparticle-mediated ocular delivery systems for small-molecules at preclinical and clinical stages, there only few for proteins and peptides which are at early stages of development. A short fragment of antiangiogenic pigment epithelium-derived factor (PEDF), was exhibited to be released from PLGA nanospheres over 40 days in vitro, although 75% of the entrapped PEDF was released in the first 10 days [147]. The surface charge of the nanoparticles also plays a crucial role in ocular penetration. One study demonstrated higher diffusion of anionic human serum albumin based nanoparticles in the vitreous relative to cationic particles [148]. Such negatively charged nanoparticles may be utilized to deliver positively charged proteins and peptides as

demonstrated by successful delivery of IgG using anionic gold nanoparticles to the photoreceptor cells and the RPE by subretinal injection [149]. Nanoparticle-mediated expression of natural antiangiogenic factors and pathway regulators offer great therapeutic potential in neovascular disorders. Plasminogen kringle 5 (K5), an 80-amino-acid proteolytic fragment of plasminogen loaded into PLGA nanoparticles exhibited reduced CNV areas and vascular leakage for at least 2 weeks in CNV models suggesting sustained antiangiogenic properties [150, 151]. Another study aimed to down regulate Wnt signaling and ocular neovascularization by increasing very low-density lipoprotein receptor extracellular domain (VLN) expression. A substantial and sustained VLN expression was achieved in cultured cells and retina for ≥4 weeks by encapsulating VLN plasmid in PLGA nanoparticles [152, 153]. Development of core-shell nanoparticles for encapsulating both hydrophobic and hydrophilic cargo [154], PEGylation for prolonging nanoparticle circulation and enhancing tissue penetration, functionalization for stimuli-responsive targeting (Fig. 14) and delivery of nanoparticles in biocompatible gels are some of the future strategies for controlled long term delivery of biotherapeutics. Our laboratory has extensively worked on encapsulating various proteins and peptides including octreotide, insulin, lysozyme, IgG-Fab, IgG, bevacizumab and catalase in a novel patented block copolymer. Our group has demonstrated successful long-term in vitro release of these macromolecules for several weeks to few months (~12 weeks) after suspending such drug-loaded nanoparticles in thermosensitive gels [155–158].

Nanofiber based systems are also being extensively explored due to their potential in generating self-assembling peptide nanofibers and peptide amphiphiles (PA). Recently, a group of researchers have demonstrated significant inhibition of endothelial cell proliferation and migration and aberrant capillary formation by delivering LPPR peptide that binds specifically to the VEGF receptor, NRP-1 as nanofibers. Furthermore, subconjunctival injection of LPPR-PA nanofiber expressively inhibited corneal neovascularization in rat model (81.3%) compared to bevacizumab (51.2%) on day 14 indicating its effectiveness in treating angiogenesis-related disorders [159].

6.2.1. Lipid based nanoparticles—Lipid based nanoacarriers including liposomes, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have been utilized as a colloidal system for controlled drug delivery. Liposomes are non-covalent aggregates that present low antigenicity and toxicity. Encapsulation of biopharmaceuticals in liposomes is commonly achieved through dehydration-rehydration method. Although this method yields high association efficiency without utilizing organic solvents and sonication, high developmental cost and particle size instability restricts its wide application. Intravitreal injection of liposomes encapsulated bevacizumab has been reported to be well tolerated through 42 days in rabbits and provided 1.5 times higher drug concentrations in the vitreous for >6 weeks [162]. Annexin A5 associated liposomes were exposed to generate 127 ng/g and 18 ng/g concentrations of bevacizumab in rat and rabbit eyes respectively after 2 hours post topical administration [163]. Furthermore, cationic liposomes offer an additional advantage of greater corneal drug absorption by increasing drug residence time through ionic interactions as shown by Cortesi et al.[164]. Li and group conjugated peptide ATWLPPR to immune-nano-liposome (INL) to deliver PEDF as a targeted therapy for CNV.

PEDF-loaded INLs significantly decreased CNV areas in rat models without binding to normal choroidal vessels [165].

SLNs are composed of biocompatible and physiological solid lipids and offer various advantages including avoidance of organic solvents for formulation, improved physical stability, targetibility, controlled release and easy scale-up. However, low drug-loading, burst-effect and rapid elimination by mononuclear phagocytic system (MPS) are some of the drawbacks of SLNs. Chetoni and co-workers demonstrated therapeutic concentrations of an antibiotic, tobramycin was achieved 3 hours in the retina and vitreous following topical and parentral SLN administrations [166, 167]. Cyclosporine-A loaded chitosan based SLNs have shown promising in vitro results with high permeation and biocompatibility in rabbit corneal endothelial cells [168]. To overcome the limited drug-loading and expulsion during phase modifications and higher water content of SLN aqueous dispersions, NLCs have been developed. NLCs are composed of highly disordered solid and liquid lipids and can provide better drug protection and entrapment efficiency in comparison to SLNs [169, 170]. Both SLNs and NLCs have shown potential in delivering small molecules to ocular tissues [171–173]. However, efficiency in delivering protein and peptide based biopharmaceuticals to ocular tissues has not been fully exploited and requires further investigation. A great deal of research has been carried out on stimuli-responsive lipid based nanocarriers for various complications (Fig. 15), and with improved design and technology such stimuli-responsive systems may become feasible for ocular delivery as well.

Niosomes are self-assembling nanovesicles composed of non-ionic surfactants that behave exactly like liposomes in vivo [177]. Although development of niosomes is still in its infancy, its potential pertinence to many therapeutic agents including small molecules, proteins and peptides can be exploited for various diseases.

6.2.2. Polymeric micelles—Polymeric micelles represent a class of nanocarriers that are composed of amphiphilic polymers which self-assemble in aqueous media to form organized supramolecular structures. Micelles have been actively studied as carriers for ocular delivery of small molecule drugs by our and other laboratories [178]. We have developed a nanomicellar formulation of cyclosporine which generated corneal concentration of 828.25 ng/g, 1 hour post single topical administration and retina/choroid concentration of 53.7 ng/g after multiple administration of 0.1% cyclosporine nanomicellar formulation [179]. In addition, triblock copolymer based positively charged micellar formulation has exhibited to prolong cyclosporine in vitro release and enhance corneal permeation in C57BL/6 mice [180]. Indeed, several micellar formulations are currently undergoing clinical trials including Seciera® (Sun Pharmaceuticals), the formulation developed by our laboratory, which just completed clinical Phase III trial for the treatment of dry eye disease. A similar nanomicellar formulation of voclosporin, VOS® (Merck Animal Health) is under clinical development for the treatment of canine dry eye syndrome.

There are fewer examples of micelles for delivering biopharmaceuticals to ocular tissues which are currently under development [181]. Synthesis and preparation of anti-Flt1 peptide-HA conjugates in the form of micelle, demonstrated to increase stability, residence time and bioavailability of anti-Flt1 peptide over 2 weeks in retinal neovascularization and

diabetic retinopathy induced rat model [182, 183]. These anti-Flt1 peptide-HA conjugate micelles were further employed to encapsulate genistein, an inhibitor of tyrosine-specific protein kinases as a combination therapy for ocular neovascularization. The micelles exhibited a sustained release for longer than 24 hours with potent inhibitory effect on vascular hyperpermeability and corneal neovascularization [184].

New ocular delivery technologies such as utilizing micelles as nano-scale microbubble precursor which can be converted into microbubble upon heating or ultrasound irradiation has got tremendous scope for ocular delivery (Fig. 16A). In addition, stimuli-responsive self-assembled intelligent polymeric vesicles capable of elucidating and biomimicking the biological activities of the lipid bilayers can offer controllable small-molecule and biopharmaceutical delivery (Figs. 16B and C).

6.2.3. Dendrimers—Dendrimers constitute branched, layered architectures composed of synthetic polymers that show promise as nanocarriers in several biomedical applications. The unique branched topologies of dendrimers confer properties completely different from linear polymers. Dendrimers can be divided into different generations (G-1, G-2, G-3) based on the size, number of branches and end groups at the terminal (Fig. 17A). A dendrimer can be composed of any type of polymer which can determine its solubility, stability and biological activity. Some of the commonly used dendrimers are based on polyamidoamines, polyamines, polyamides (polypeptides), poly(aryl ethers), polyesters, carbohydrates and DNA. Among these, polyamidoamine (PAMAM) based dendrimers are most commonly used and commercially available. Unlike linear polymers, the multivalent property of dendrimers provide a means to achieve high concentrations of payloads including small-molecules, biopharmaceuticals and imaging agents. The molecular weight and surface charge of dendrimers also play a crucial role in determining tissue accumulation profiles, drug release rates (from the polymer) and elimination rates. While, high molecular weights of dendrimers (>~40 kDa) prevent rapid clearance, uncharged or negatively charged surface limit nonspecific interactions [188]. In addition, numerous end groups offer a way to precisely control functionality with multiple copies of drugs, chromophores, peptides, proteins and multivalent ligand density. Such surface modifications can not only strengthen ligand-receptor binding and improve the targeting of attached components but can also accelerate dendrimers stimuli-responsive activity [189].

PAMAM dendrimers as drug delivery vehicles for small-molecules have shown improved biological response, tolerability and lower clearance from ocular surface indicating its utility as an eye drop formulation [190]. A few studies have also confirmed the delivery of therapeutic peptides and proteins using different conjugation techniques with dendrimers (Figs. 17B, C and D) [191–194]. However, targeted delivery of protein and peptide based biopharmaceuticals with dendrimers for ocular complications has not been reported so far. In addition, while there are several in vitro efficacy studies demonstrating the effectiveness of dendrimers as nanocarriers, only a few in vivo efficacy studies exist. Nonetheless, despite several advantages of dendrimers, multistep syntheses, high preparation costs and inadequate quality control assays prohibited significant advancement of dendrimers from the laboratory to the clinic.

6.2.4. Nanowafers—Nanowafers are tiny transparent circular discs that are composed of various polymers including poly(vinyl alcohol) (PVA), polyvinylpyrrolidone (PVP), (hydroxypropyl)methyl cellulose (HPMC), and carboxymethyl cellulose (CMC). These are generally applied with a fingertip on ocular surface and can withstand constant blinking without being displaced unlike topical eye drops. Nanowafers consist of arrays of drug-loaded nanoreservoirs which releases the cargo in a highly controlled manner for a few hours to several days (Fig. 18). The synergistic action between the polymers and the loaded drug leads to slow drug release thus enhancing drug residence time and subsequent absorption into ocular tissues. Importantly, at the end of stipulated drug release time, the nanowafer dissolves and fades away thus rendering ocular surfaces free of polymers. Yuan and group for the first time demonstrated the sustained release and enhanced corneal permeability of doxycycline-loaded PVA nanowafer over 24 hours in mice. The same group reported the efficacy of such PVA fabricated nanowafer loaded with axitinib for treating CNV in a murine ocular burn model [196, 197]. This smart platform integrating nanofabrication as well as drug delivery technologies has been able to establish itself further by demonstrating sustained delivery of dexamethasone and cysteamine for effective treatment of dry eye disease and corneal cystinosis respectively [198–200]. These results provide a strong rationale for their translation and clinical application in biopharmaceuticals delivery.

6.3. Biodegradable and non-biodegradable polymeric implants

Biodegradable implants can overcome the challenges of particulate systems such as suspension, reconstitution, quantification and manufacturing complexities. In addition, higher drug loading due to larger size and smaller surface-to-volume ratio in comparison to particulate systems, allows prolonged drug release with reduced or minimal burst release. Utilization of additional materials including PEG400 and blends of block co-polymers and PLGA have shown to provide prolonged release of protein drugs with reduced initial burst [172]. Biodegradable implants are capable of sustaining the release from a few days to several months. The devices are introduced directly in the vitreous or onto the sclera by minor surgical techniques [201]. Similar to polymeric particulate systems, biodegradable implants also suffer from poor stability and release rates. Additionally, implantation of such devices requires skilled professional execution leading to their towering price tags. Ozurdex (Allergan®) is one such biodegradable PLGA based implant approved by FDA for macular edema and noninfectious posterior uveitis [202]. The PLGA copolymer matrix releases loaded dexamethasone (0.7 mg) in the vitreal cavity for a period of 6 months [203]. A similar formulation has been evaluated for the delivery of brominidine tartrate in clinical trials (NCT02087085). Surodex (Oculex Pharmaceuticals, Sunnyvale, CA) and Verisome (Icon Biosciences Inc., Sunnyvale, CA) are a few other biodegradable implants that are currently undergoing clinical trials [204]. To date no ophthalmic biodegradable implant for biopharmaceutical drugs has been approved. However, a few biodegradable implants have demonstrated their potential in preclinical studies. An implant loaded with human recombinant tissue plasminogen activator (t-PA) has shown to release t-PA at a rate of 0.5 μg/day for 2 weeks in the vitreous [205].

In contrast, non-biodegradable implants are composed of a core drug reservoir and a semi-permeable membrane allowing continous drug release for up to several months to years. While biodegradable implants are more susceptible to burst and non-linear release kinetics, non-biodegradable implants release drugs following zero-order kinetics. However, non-biodegradable implants requires to be removed or refilled following drug reservoir depletion. Several non-biodegradable implants have been clinically approved including Vitrasert®, Retisert® (Bausch & Lomb, Rochester, NY), Iluvien® (Alimera Sciences, Inc, Alpharetta, GA) for the delivery of small molecule drugs to the vitreous [206–208]. However, large size with the exception Iluvien, surgical procedures and complications such as retinal detachments are a few drawbacks of non-biodegradable implants. Currently, nonbiodegradable implants for biopharmaceutical drugs have not yet reached the market. An osmotic pump, implanted in the subcutaneous space and connected to the sclera using a brain infusion kit has been reported to deliver IgG for 28 days [209]. Ranibizumab port delivery system (PDS) (Genentech, CA) is another non-biodegradable implant that has shown promise in delivering therapeutic concentrations of ranibizumab into the vitreous over an extended period of time and is currently undergoing Phase II clinical trials (NCT02510794). Implants with suitable design, smaller size, minimal surgical procedure, maintenance of biopharmaceutical stability and prolonged release[210] may serve as an attractive system for biopharmaceuticals delivery to ocular tissues.

6.4. In situ gelling formulations

In situ-forming gels are low-viscosity polymeric solutions that undergo phase transition to form a gel following stimulus. Such phase transition can be mediated by changes in temperature, pH, light and ionic composition. Various in-situ gelling systems including chitosan, poloxamer, hydroxypropylmethylcellulose and polycaprolactone have exhibited safe use as ocular depot systems. Topical application of in-situ gelling systems not only aids in increasing precorneal drug residence time but also offers increased stability and bioavailability in ocular tissues. Although, injectable solutions of PLGA in N-methyl pyrollidone and sucrose acetate isobutyrate are known to prolong biopharmaceutical drug delivery, extremely poor permeability of macromolecules across corneal epithelium may present a major obstacle. Till now, several injectable in-situ gelling formulations for ocular delivery of small-molecules have been studied. In fact, such in-situ depot systems are being developed for biopharmaceuticals delivery as well [211]. An example of light activated in-situ gelling system that shows promise is the delivery of bevacizumab in the suprachoroidal space for 60 days in rats [212]. In another study, intravitreal injection of bevacizumab loaded thermosensitive gel exhibited an extended release profile over 18 days in vitro [213] and anti-angiogenic effects in 3-D cultures [214]. Polysaccharides cross-linked hydrogels have shown to sustain bevacizumab release for 3 days with initial burst release for 4 hours [215]. Similarly, Lovett and co-workers demonstrated the ability of silk hydrogels in sustaining bevacizumab release for 3 months in experiments in vitro as well as in vivo in Dutch-belted rabbits [216]. Recently, a reverse thermal gelling system based poly(ethylene glycol)-poly-(serinol hexamethylene urethane) (ESHU) demonstrated sustained release of bevacizumab over 9 weeks in vivo [217]. The unique gelling properties of such depot systems can be optimized to achieve long-term release of biopharmaceuticals for several weeks to few

months. In Table 5, we highlight some of the advantages and limitations of various ocular delivery systems for biopharmaceuticals.

6.5. Delivery using cell penetrating peptides (CPP)

CPPs have shown great potential to quickly translocate attached molecules through the cell membranes into mammalian cells both in vitro and in vivo. Such CPP-conjugated nanocarriers are generally taken up by energy-dependent endocytosis (or macropinocytosis) after electrostatic interactions [218] and can enhance the delivery of small-molecule drugs and biopharmaceuticals to ocular tissues. In this regard, 86-mer trans-activating transcriptional activator (TAT) from HIV-1 has been widely applied to achieve targeted delivery [219]. However, one of the major obstacles that still remains unresolved is the lack of selectivity of TAT. To overcome such a short coming, a novel peptide for ocular delivery (POD, $GGG[ARKKAAKA]_4$) has been recently developed that is capable of delivering protein and peptide based biopharmaceuticals specifically to their target side thus reducing unwanted side effects. Moreover, POD administered topically has shown to reach the dura of the optic nerve within 45 minutes indicating its effectiveness in treating optic nerve diseases. POD also exhibited bacteriostatic activity by reducing bacterial colony number significantly at lower concentrations [220, 221]. Various CPPs of human proteins are being extensively exploited to overcome immunogenicity, poor serum stability and toxicity arising from CPP sequences of non-human origin [222].

Stapled peptides, a class of helical peptides that have recently gained interest focuses on improving membrane penetration of peptides by incorporating modifications such as α-methylation and hydrocarbon based macrocyclic bridging features or mutagenesis to improve hydrophobicity and conformational stabilization of the helix. It has been observed that both noncovalent and covalent constraints to stabilize long peptide sequences have resulted in enhanced binding [223]. However, noncovalently constrained constructs are rapidly proteolyzed and covalently constrained peptides bearing labile cross-links (for example, disulfides and amides) are also vulnerable to proteolysis [224]. Several studies have shown peptide constraints through installation of thioether, lactam or triazole cross-links, or helix promoting non-natural amino acids, have reinforced structure and enhanced peptide-antibody binding affinity [225–227]. Bird and group performed all-hydrocarbon cross-link by olefin metathesis of installed non-natural amino acids bearing olefin tethers to determine the binding of stabilized α-helices of membrane-proximal external region (SAH-MPER) peptide of gp41 with broadly neutralizing anti-HIV-1 antibodies (4E10 and 10E8). The modified SAH-MPER exhibited high-binding affinity towards 4E10 and 10E8 and markedly enhanced protease resistance (Fig. 19) [228]. Such modulations of stapled peptides to enhance protein-protein interactions have been successfully employed in cancer treatment as well. This has led the entry of a stapled peptide, ALRN-5281 (growth-hormone-releasing hormone agonist, Aileron Therapeutics) into clinical trials [229]. Applications of stapled peptides in the context of ocular delivery have not been explored so far. This strategy may prove to be promising for the treatment of ocular complications [230].

6.6. Delivery using living cells

Biomedical applications of cell based drug delivery systems have opened up new perspectives of using our own cells for therapeutic purposes. Such application in improving pharmacokinetic profiles or generating sustained release depots have progressed to clinical evaluations. Encapsulated cell technology (ECT) is one such advancement which utilizes entrapment of genetically modified cells within a semipermeable membrane in order to isolate them from host body. The genetically engineered cells are designed in such a way that allow continuous production of therapeutic proteins. The semipermeable membrane apart from protecting the encapsulated cells, allows passage of nutrients into the cells, while providing sustained release of therapeutic proteins from the implant. NT-501 (Renexus, Neurotech Pharmaceuticals), which aims to protect the retina from degeneration by producing ciliary neurotrophic factor (CNTF), provides sustained delivery of CNTF from the implant encapsulating genetically engineered human retinal pigment epithelial (RPE) cells [232]. A similar ECT, NT-503 has been developed that secretes soluble VEGF receptor protein (Fc-Fusion protein) for a prolonged period of time and has showed significantly higher (20–30 fold higher) VEGF neutralization [233, 234]. ECT seems to be an attractive modality and a better control over the release rate may prove to be exciting in achieving long-term ocular delivery of biopharmaceuticals. Some of the controlled-release systems under investigation for protein and peptide therapeutics indicated for ocular complications are detailed in Table 6.

6.7. Delivery using minimally invasive microneedles and iontophoresis

Microneedles (MNs) as evident from some of the previous examples, have gained wide application due to their potential in circumventing epithelial transport barrier and conjunctival clearance mechanism, and at the same time minimizing retinal damage. Ocular MNs are primarily based on passive [249] and active [125, 250, 251] delivery approaches. Passive MNs typically consist of arrays of solid MNs coated with drug formulations that are intended to dissolve within minutes of insertion, followed shortly by removal of the device. Such simplified nature of passive MNs render them more advantageous for routine clinical use and commercial feasibility. Microneedle pens developed by Song and group have demonstrated to inhibit corneal neovascularization by delivering sunitinib malate in vivo [249]. Nevertheless, the limited drug loading capacity of such devices may constrain their potential for clinical translation. Aimi and coworkers have made an effort to address this limitation by designing titanium-based MNs (Ti MNs) through titanium deep reactive ion etching (Ti DRIE) process. The unique through-thickness fenestrations (i.e. windows) of Ti MNs acts as drug reservoirs and allows increase drug carrying capacity relative to solid MNs [252]. The same group reported a uniform drug deposition and fenestration filling techniques that increased drug carrying capacity of fenestrated Ti MNs five-fold relative to solid MNs of comparable size. Such fenestrated MNs along with micro- or nanoparticle-entrained coatings may hold potential for demonstrating sustained biologics delivery as well and thus reduced treatment frequency. Fig. 20 illustrates scanning and fluorescence micrographs of different types MNs.

Iontophoresis has been exploited for many years as a non-invasive technique for ocular drug delivery. Although a series of small molecules including ciprofloxacin hydrochloride (ocular

infection)[254], gentamicin (pseudomonas keratitis) [255], dexamethasone phosphate [256], methylprednisolone (posterior segment inflammation) [257], carboplatin (retinoblastoma) [258], and methotrexate (inflammatory diseases and intraocular lymphoma) [259] have been delivered successfully, ocular delivery of proteins and peptides using this technique have been rarely explored. A better understanding of electric current application in ocular tissues and advanced designing of devices and probes adapted to the application site may yield efficient intra-ocular penetration of proteins and peptides based therapeutics as well [260]. Zhang and coworkers have recently developed one such flexible ocular iontophoretic device that can be placed under the eyelid and deliver ions through a small area on the eyeball, reducing tissue damage caused by drug during ion penetration [261].

7. Conclusion

Despite the major hurdles in ocular delivery of proteins and peptides, technological breakthroughs in formulation, delivery approaches and manufacturing methods have facilitated the growth and improvement in the biopharmaceutical market. Some of the work with the delivery of biopharmaceutical drugs have shown encouraging results. However, many needs remain unmet for the delivery of relatively smaller biologics, and greater challenges keep arising for developing formulations for larger biopharmaceutical drugs. Current biopharmaceuticals suffer from poor intracellular delivery leading to low ocular bioavailability, reduced stability (including storage, handling and administration), incompetent formulation development strategies and scalability and high manufacturing costs. Developing new biomaterials for effective protection of proteins and peptides and improving intracellular delivery by identifying target-site specific receptors will significantly improve biopharmaceutical delivery.

Another area for future progress is the development of novel formulation and delivery approaches for biopharmaceutical drugs. While extraocular delivery strategies including tropical eye drops and periocular injections have exhibited poor bioavailability and limited targeting, intraocular strategies such as intracameral and intravitreal injections are highly invasive. Efforts should be made to reduce the frequency of intraocular administration (e.g. novel controlled release formulations) and develop new methods or devices (e.g. through non-parenteral routes) that are non-invasive in nature. Expansion of therapeutic target space that are not accessible by the routinely used biologics or small-molecules can pave the way to unexplored therapeutic opportunities. Future research should focus on non-invasive controlled intraocular delivery of highly stable formulations of proteins and peptides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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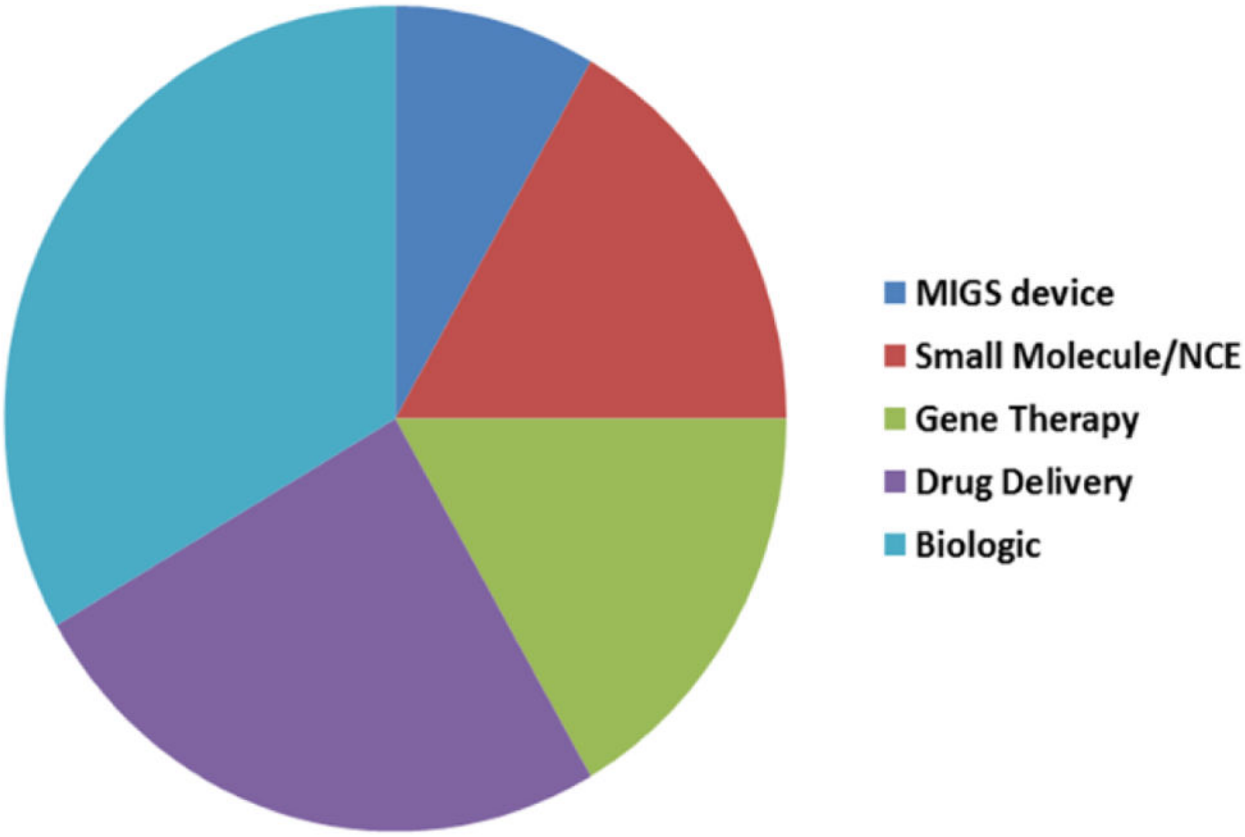

Fig. 1.
Numbers of Phase 3 products by technology type for ophthalmic indications (Till Nov., 2015): MIGS (minimally invasive glaucoma surgery); NCE (New chemical entity)

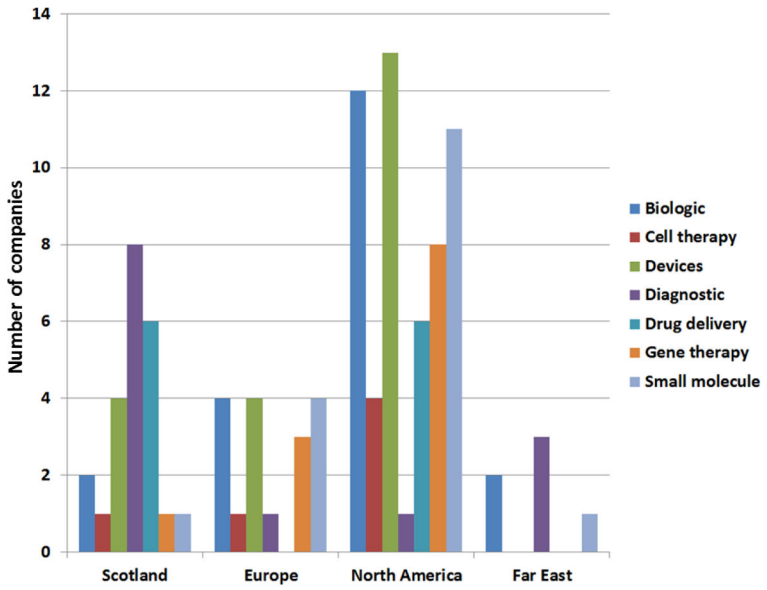

Fig. 2.

Number of companies classified by technology as well as global areas for ophthalmology market: This analysis does not include multinational companies, as these entities cannot be defined by a single technology and any one country. Note that the classification “Europe” excludes Scotland to avoid double counting.

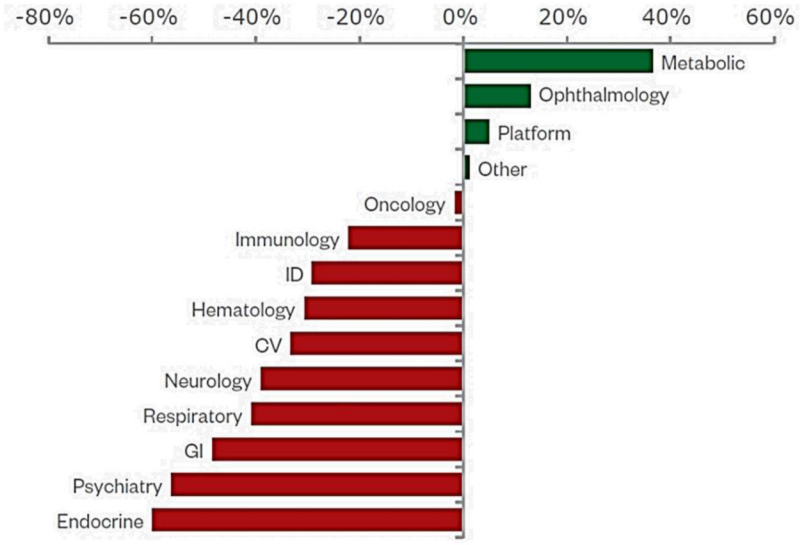

Fig. 3.
Novel drug R&D venture funding by disease area, 2004–2008 vs 2009–2013

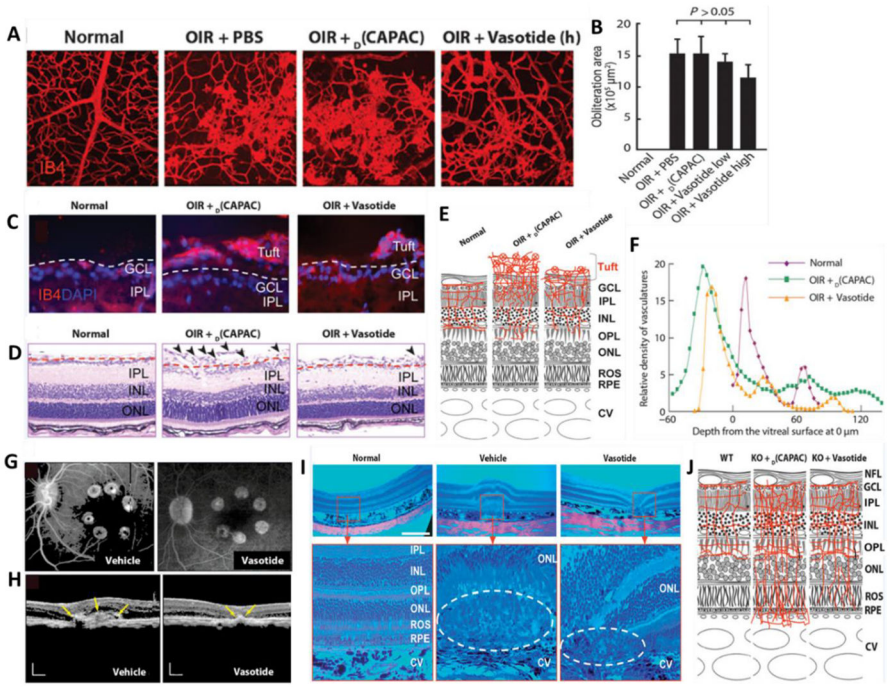

Fig. 4.

(A) Effect of intraperitoneally injected therapeutic Vasotide peptide on the vasculature and tuft formation in 19 days old (P19) normal and oxygen-induced retinopathy (OIR) mice in comparison to PBS and control D(CAPAC) peptide; (B) Quantitation of tuft areas in wild-type (WT) mice given treatment groups as eye drops; (C) Cryostat sections of vascular tufts extending from the retina into the vitreous with IB4-stained vessels in red and DAPI (4′,6-diamidino-2-phenylindole) counterstained nuclei in blue at P19. Pathological tuft formation is shown above the dashed lines, and reduced vessel formation within the inner retina is shown below the dashed lines; (D) Paraffin sections showing tuft formation above dashed lines and retinal layers below dashed lines; (E) Diagram of the vasculature in different regions of the retina; (F) Quantitation of percent blood vessel area at 4-mm intervals summed through the full retina on a relative scale; (G) 6-min fluorescein angiograms for monkeys treated with eye drops at 29 days after laser-induced photocoagulation; (H) OCT images from monkeys given eye drops at 29 days after the laser-induced lesion. Yellow arrows indicate CNV complex boundaries; (I) H&E-stained monkey retinas at low (upper row) and high (lower row) magnifications showing eosin red–stained vacuolated fibroblast layer outside of the choroid in the upper row. Red boxes indicate macular region; dashed ovals indicate the RPE and ROS zones 29 days after laser-induced lesioning; (J) Diagram showing vascular differences in the retinas of WT mice and vldlr-null (KO)mice treated with treatment groups. NFL, nerve fiber layer; GCL, the ganglion cell layer; IPL, the inner plexiform layer; INL, the inner nuclear layer, OPL, the outer plexiform layer; ONL, outer nuclear layer; ROS, rod (and cone) outer segments; RPE, the retinal pigment epithelium; CV, choroidal vessels. Reprinted from [25].

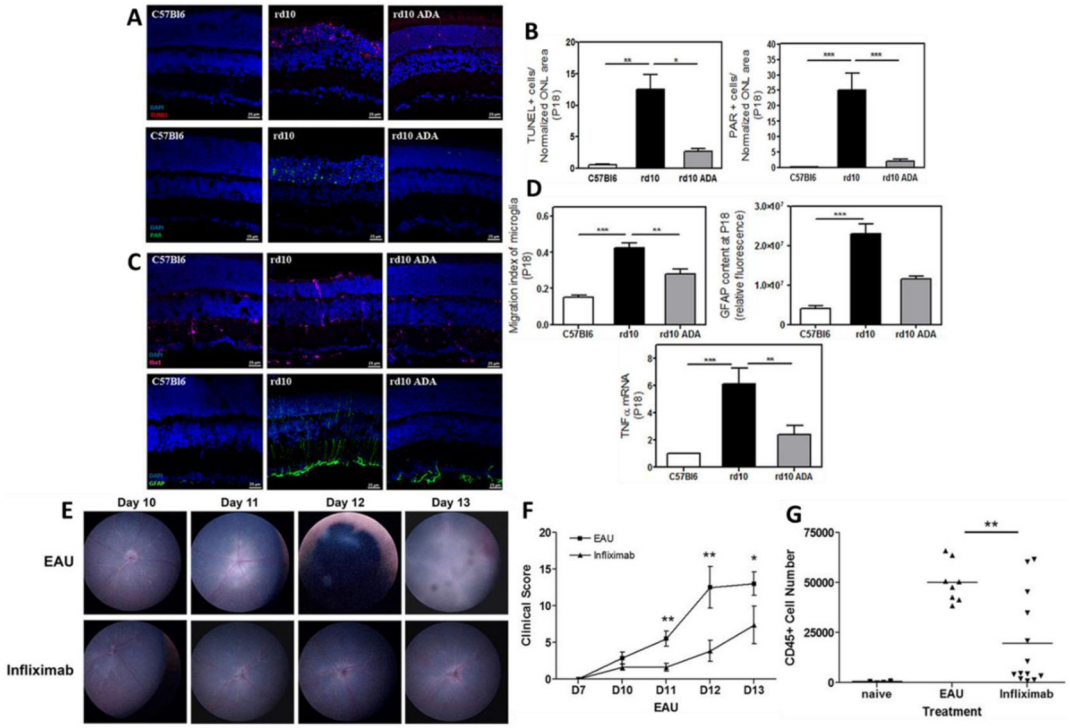

Fig. 5.

(A) Photomicrographs of retinal sections showing significant reduction in photoreceptor cell death in the murine model of human autosomal recessive retinitis pigmentosa, the rd10 mice at postnatal day (P) 18 after Adalimumab (ADA) treatment in comparison to control (C57Bl6) (TUNEL-stained sections revealing dead photoreceptors and PAR content in DAPI-counterstained sections); (B) Bar graph illustrating the effect of ADA on the number of TUNEL-positive nuclei and nuclear poly (ADP) ribose (PAR)-positive cells; (C) Photomicrographs of retinal sections showing reactive gliosis amelioration by ADA in the rd10 mouse retina at P18 (Iba1-labelling to visualize microglial cells and GFAP content in DAPI-counterstained sections); (D) Bar graphs illustrating the effect of ADA on migration index of microglia, the corrected fluorescence of GFAP content and TNFα gene expression; (E) Topical endoscopic fundal imaging (TEFI) images showing intravitreal administration of infliximab suppresses experimental autoimmune uveitis (EAU) in comparison to vehicle control; (F) combined total disease scores demonstrating the difference in clinical disease progression between treatment groups. In EAU control eyes typical disease progression with signs of raised optic disc, vasculitis and severe inflammation; In infliximab treated eyes, only raised optic disc and initial signs of vasculitis are evident; (G) Graph showing total CD45+ infiltrate numbers from individual eyes. Reprinted from [33, 34].

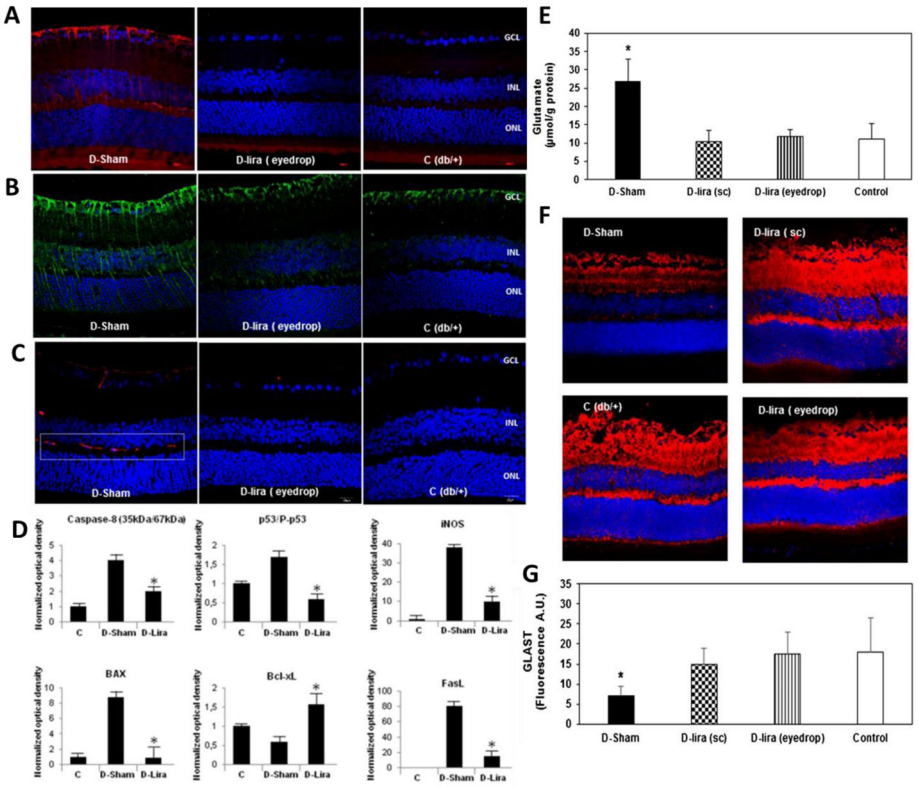

Fig. 6.

Immunofluorescence images from a diabetic mouse (D) after topical administration of GLP-1R agonist, liraglutide (D-lira eye drop) in comparison to vehicle (D-Sham) and a non-diabetic mouse (control, C; db/+). D-lira prevented disruption of the BRB and thus release of VEGF (red) (A), IL-1b (green) (B) and albumin (red) (C), most important players in the pathogenesis of the breakdown of the BRB; (D) Western blotting quantification of proteins from apoptotic (caspase 8, Bax, p53), antiapoptotic (BclxL) and neuroinflammatory (iNOS, FasL) signaling pathways; (E) Retinal concentration of glutamate measured by high-performance liquid chromatography after subcutaneous administration of treatment groups; (F) Comparison of glutamate/aspartate transporter (GLAST) immunofluorescence (red) after topical administration of treatment groups; (G) Quantification of GLAST immunofluorescence in arbitrary units (A.U.). Reprinted from [36].

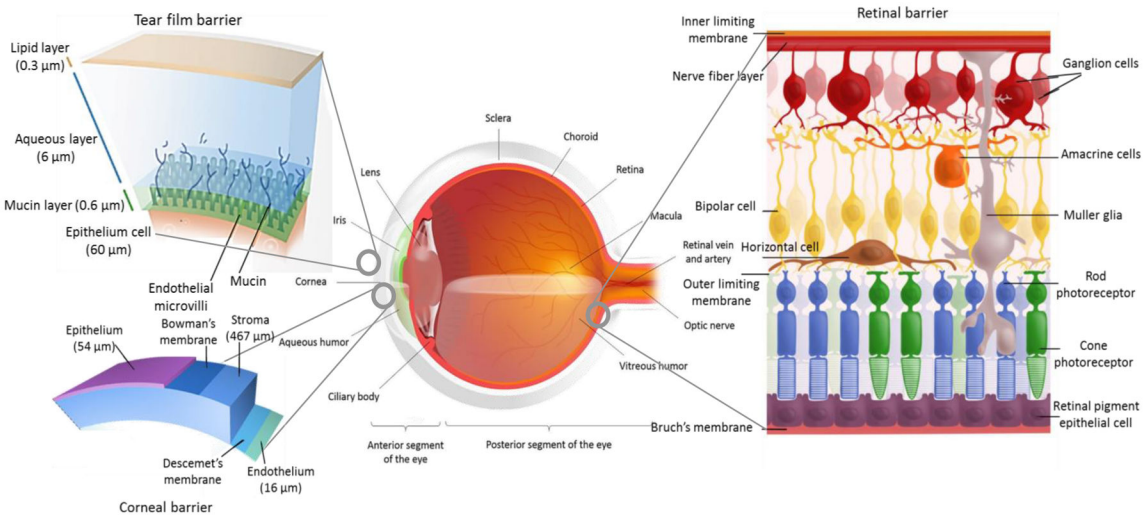

Fig. 7.

Ocular anatomy and tissue barriers. Reprinted and modified from [50].

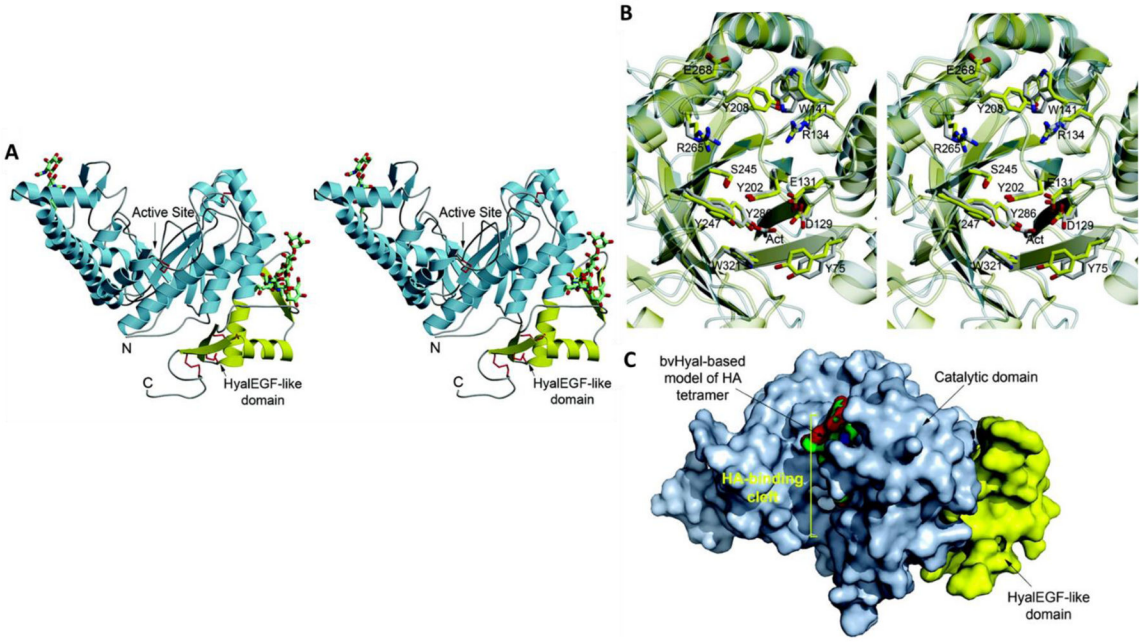

Fig. 8.

(A) Structure of hHyal-1: Stereoscopic representation of a side view. The catalytic and the HyalEGF-like domains are colored light blue and yellow respectively. Disulfide bonds are shown in red. N-linked oligosaccharides are shown as stick models with the atomic color scheme: gray, carbon; red, oxygen; blue, nitrogen; (B) Stereoscopic representation of the active site region of hHyal-1 (gray ribbon) superimposed on that of bvHyal (yellow ribbon). Selected amino acids are colored in the atomic color scheme: red, oxygen; blue, nitrogen; gray (hHyal-1) and yellow (bvHyal), carbon. (C) Molecular surface of the catalytic domain (light blue) and HyalEGF-like (yellow) domains of hHyal-1, illustrating the separation between the HyalEGF-like domain and the active site. A docked tetrasaccharide, inferred from the structure of bvHyal, is shown as a space filling model. Reprinted from [79].

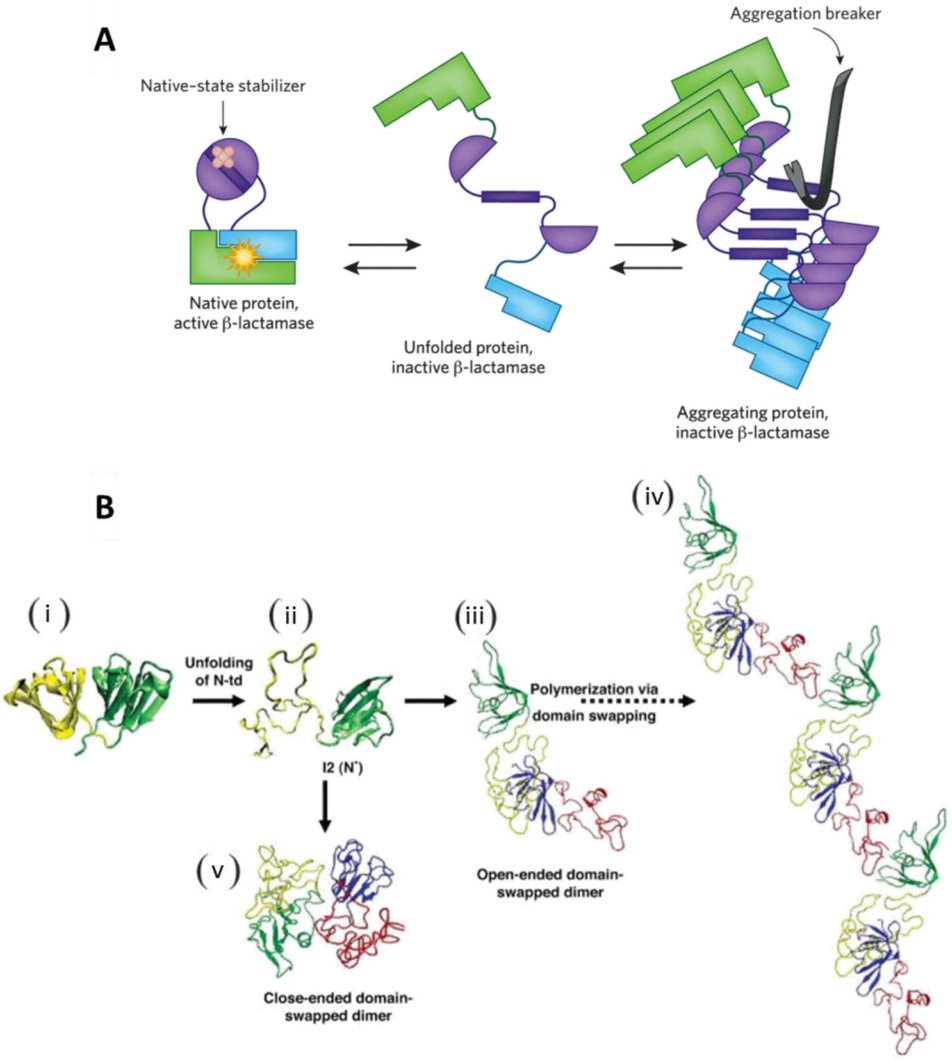

Fig. 9.

(A) Functioning of a chemical chaperone: β-lactamase function is restored when aggregation of the target protein is inhibited. This can occur either through stabilization of the native structure (left) or through inhibition of the process of amyloid self-assembly (right). Reprinted from [86]. (B) Schematic summary of human γD-crystallin (a member of crystallin families) polymerization. (i) Crystal structure of human γD-crystallin. (ii) Simulated monomeric aggregation precursor (I2), often referred as N* in the general mechanism of protein aggregation in literature. (iii) Simulated structure of open-ended domain-swapped dimer. (iv) Simulated structure of close-ended domain-swapped dimer. (v) Model of human γD-crystallin hexamer formed via domain swapping. Reprinted from [84].

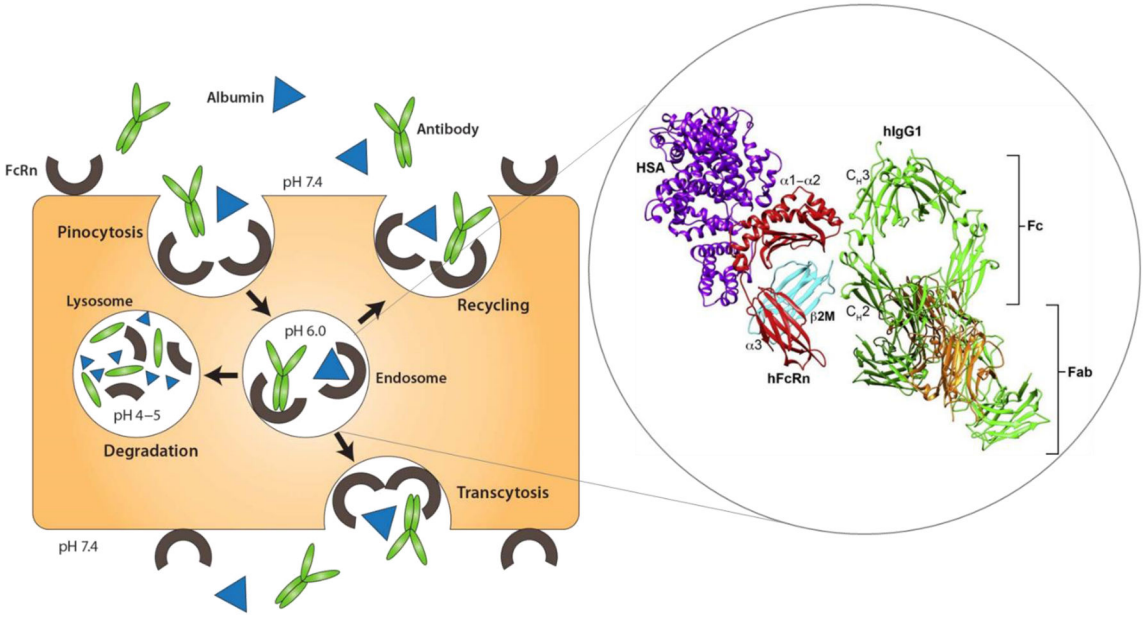

Fig. 10.

Structure of human FcRn in contact with human IgG1 (hIgG1) and human serum albumin (HAS) and FcRn-mediated recycling of IgG and albumin in vascular endothelial cells; IgG and albumin are internalized into vascular endothelial cells through pinocytosis. The pH of the endosome is 6.0, facilitating association with membrane-bound FcRn. The contents of endosomes can be processed in one of two ways: either recycling back to the apical cell membrane or transcytosis from the apical to the basolateral side. In the case of saturated receptors, excess IgG and albumin are degraded by lysosomes. Top, apical side; bottom, basolateral side. Reprinted and modified from [95].

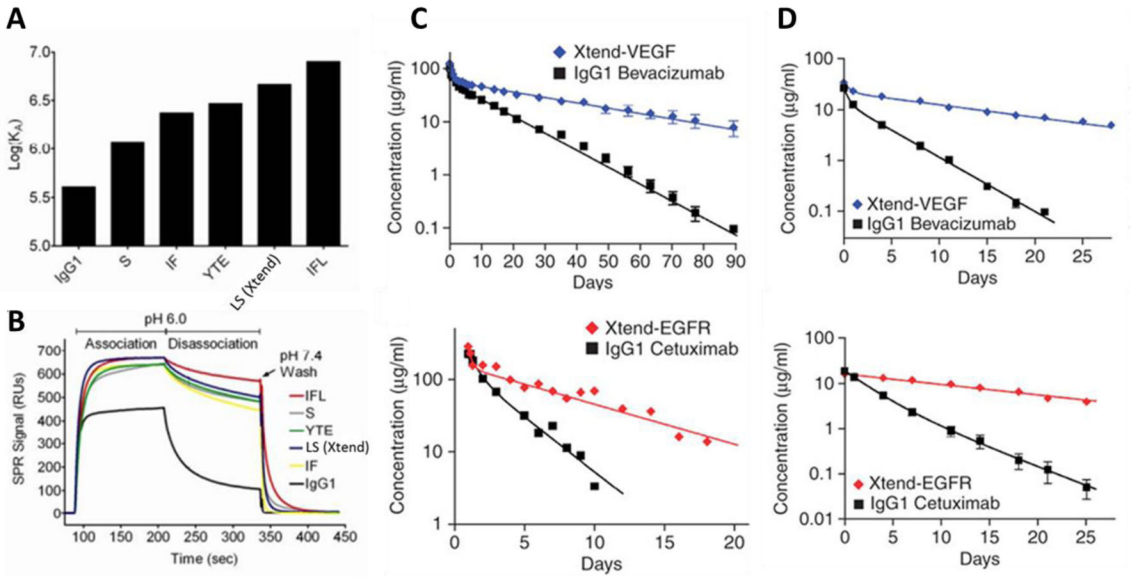

Fig. 11.

(A) The log of the equilibrium association constant KA at pH 6.0 are plotted for various engineered anti-VEGF (bevacizumab) variants demonstrating increased binding to human FcRn in contrast to parent bevacizumab native IgG1 antibody; (B) Binding sensorgrams at pH 6.0 and 7.4 of each variant; Log-linear changes in serum concentrations for anti-VEGF (bevacizumab) and anti-EGFR antibodies in cynomolgus monkeys (C) and hFcRn transgenic mice (D) demonstrating antibodies engineered for higher FcRn affinity (Xtend-VEGF and Xtend-EGFR) promotes half-life extension. Reprinted from [96].

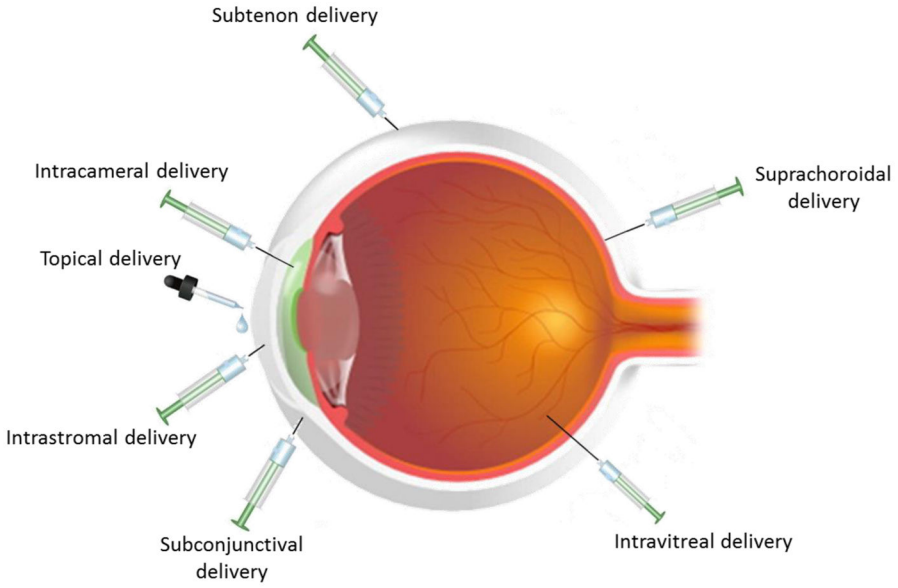

Fig. 12.

Current and emerging routes for protein and peptide delivery to ocular tissues. Reprinted and modified from [50].

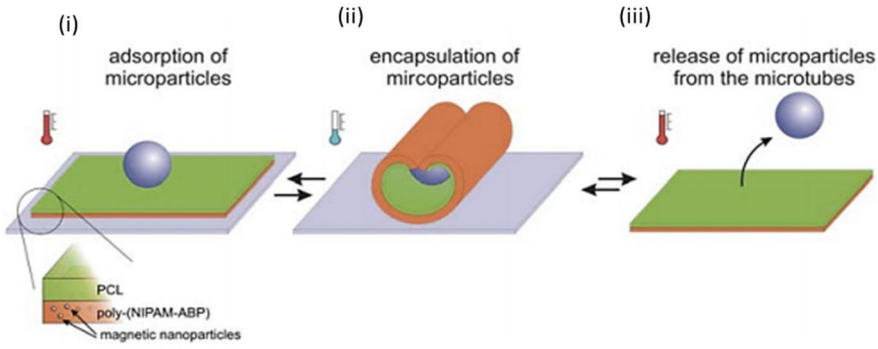

Fig. 13.

Schematic of capture and release of microparticles by self-rolling microtubes. Thin film of poly(N-isopropylacrylamide-co-4-acryloylbenzophenone)(poly(NIPAM-ABP)) and polycaprolactone (PCL) with admixed magnetic nanoparticles (i) is able to form self-rolling tube and to encapsulate microparticles at reduced temperature (ii). The particle can be released at elevated temperature when the microtube is unrolled (iii). Reprinted from [146].

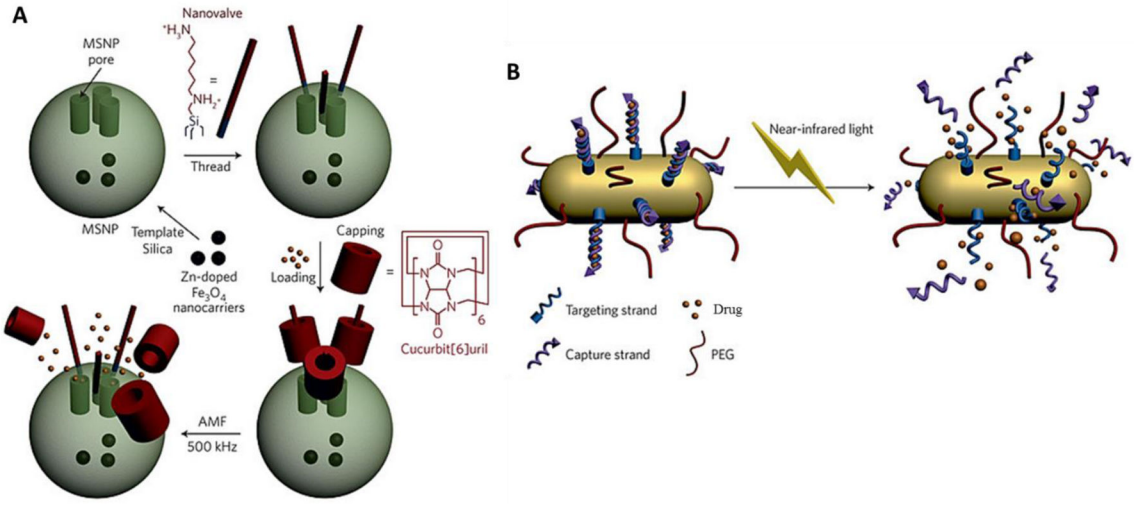

Fig. 14.

(A) Actuation mechanisms based on the heat generated by an alternating magnetic field (AMF) leading to on-demand pulsatile small molecule release from mesoporous silica nanoparticles (MSNPs): Pseudorotaxane-based nanovalves made of cucurbit[6]uril. Reprinted from [160]; (B) Light-triggered small molecule delivery: Drug delivery through the near-infrared-triggered induction of dehybridization of the DNA conjugated at the surface of gold nanorods. Reprinted from [161].

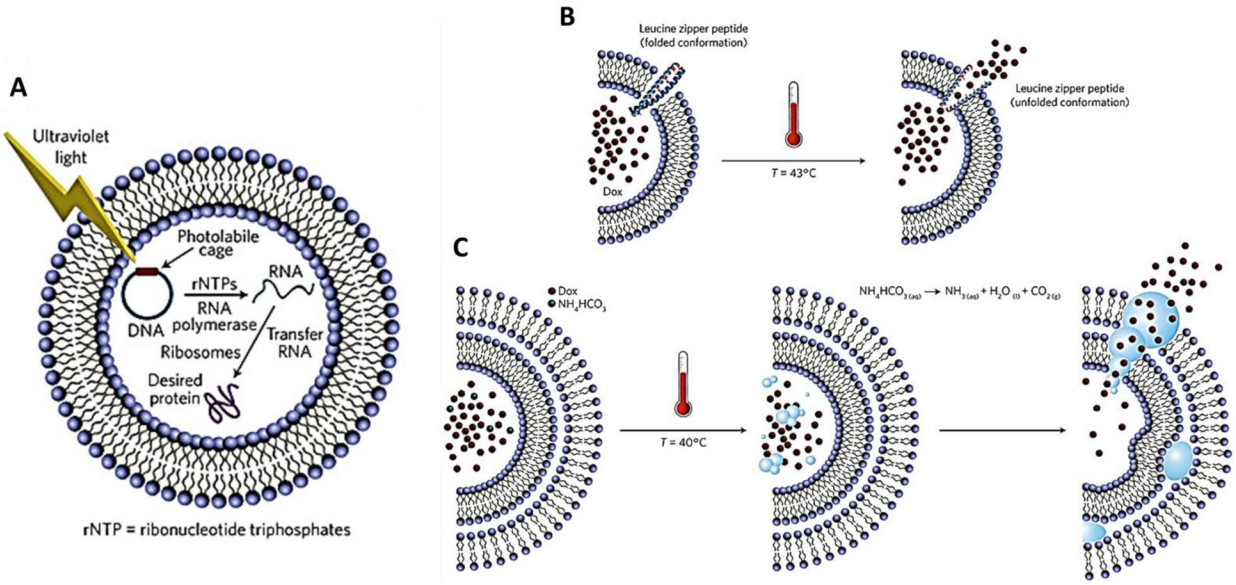

Fig. 15.

(A) Light-triggered drug delivery: Schematic representation of transcription–translation liposomal system for protein production triggered by irradiating caged DNA with light. Reprinted from [174]; (B) Temperature-based actuation mechanisms for liposomal drug delivery: The temperature-triggered unfolding of a leucine zipper peptide inserted in the membrane of a doxorubicin (Dox)-carrying liposome opens a channel through which the drug is released. Reprinted from [175]; (C) Drug-permeable pores can also be created by the temperature-triggered generation of bubbles from the decomposition of encapsulated ammonium bicarbonate. Reprinted from [176].

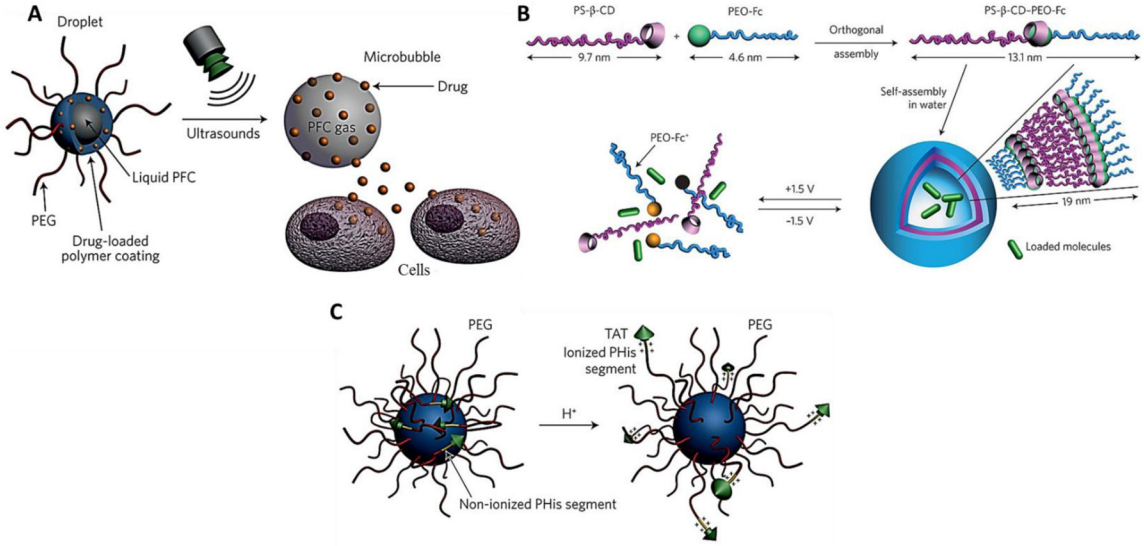

Fig. 16.

(A) Drug delivery from echogenic perfluorocarbon (PFC)-containing nanoemulsions: The delivery mechanism involves a droplet-to-bubble transition under the action of ultrasound, leading to drug transfer from the bubbles to neighbouring cells. Reprinted from [185]; (B) Voltage-responsive vesicles: Structures of polystyrene-β-cyclodextrin (PS-β-CD) and poly(ethylene oxide)-ferrocene (PEO-Fc), and representation of the voltage-responsive controlled assembly and disassembly of PS-β-CD–PEO-Fc supramolecular vesicles. Reprinted from [186]; (C) pH-sensitive nanocarriers for efficient TAT-peptide exposure: Polyhistidine (PHis)-based micelles responding to acidic microenvironments by an efficient TAT-sequence exposure following ionization of the polyhistidine segments. Reprinted from [187].

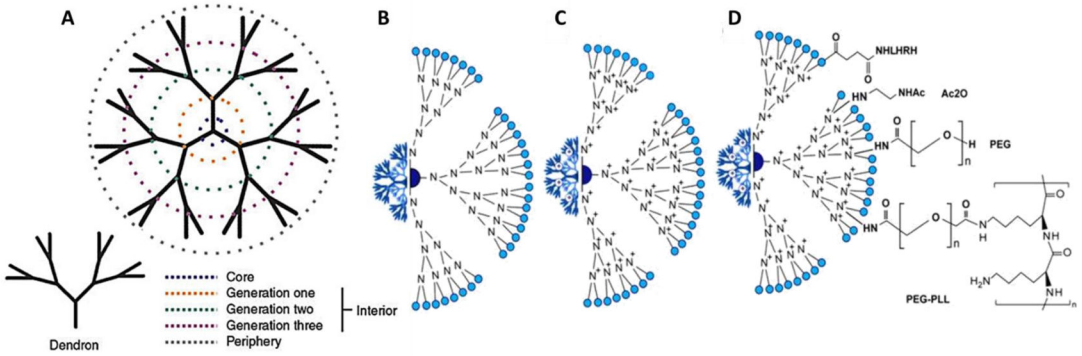

Fig. 17.

(A) Anatomy of a dendrimer: A dendrimer and dendron are represented with solid lines. The colored, broken lines identify the various key regions of the dendrimer. Reprinted from [188]; (B) Generation 4 PAMAM-OH dendrimer. (C) Internally quaternized PAMAM to form QPAMAM-OH dendrimer with inner cationic charges. PAMAM are frequently quaternized by methyl iodide (ICH3) and the terminal surface become very positive allowing the efficient electrostatic binding/loading of negatively charged backbone of siRNA. (D) QPAMAM with different surface modifications, including the addition of acetyl group by direct reaction with acetic anhydride (Ac2O), poly(ethylene glycol) (PEG) and poly-L-lysine (PLL), LHRH peptide. This addition of polymer structures such as PEG and PLL is reported to enhance the surface positive charge and circulation of PAMAM nanoparticles. While, the conjugation to LHRH peptides confers targeting ability in PAMAM based delivery applications. Reprinted from [195].

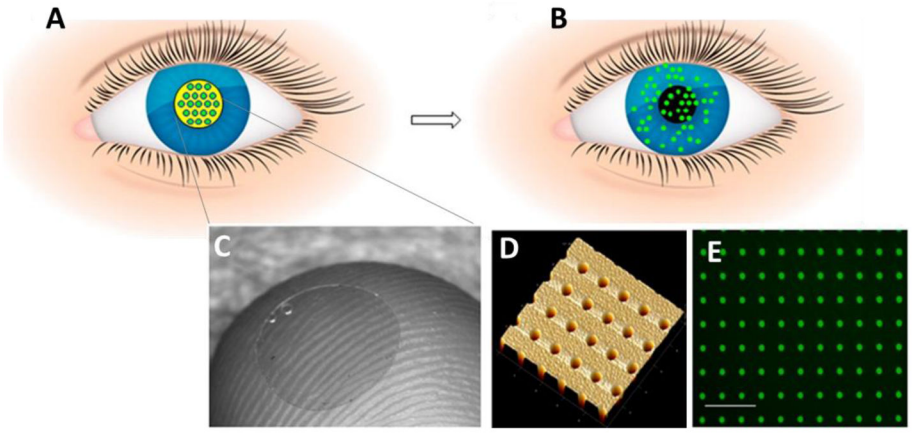

Fig. 18.

Ocular drug delivery nanowafer: (A) Schematic of nanowafer instilled on the cornea. (B) Diffusion of drug molecules into the corneal tissue. (C) Nanowafer on a fingertip. (D) AFM image of a nanowafer demonstrating an array of 500 nm diameter nanoreservoirs. (E) Fluorescence micrograph of a nanowafer filled with doxycycline (scale bar 5 μm). Reprinted from [196].

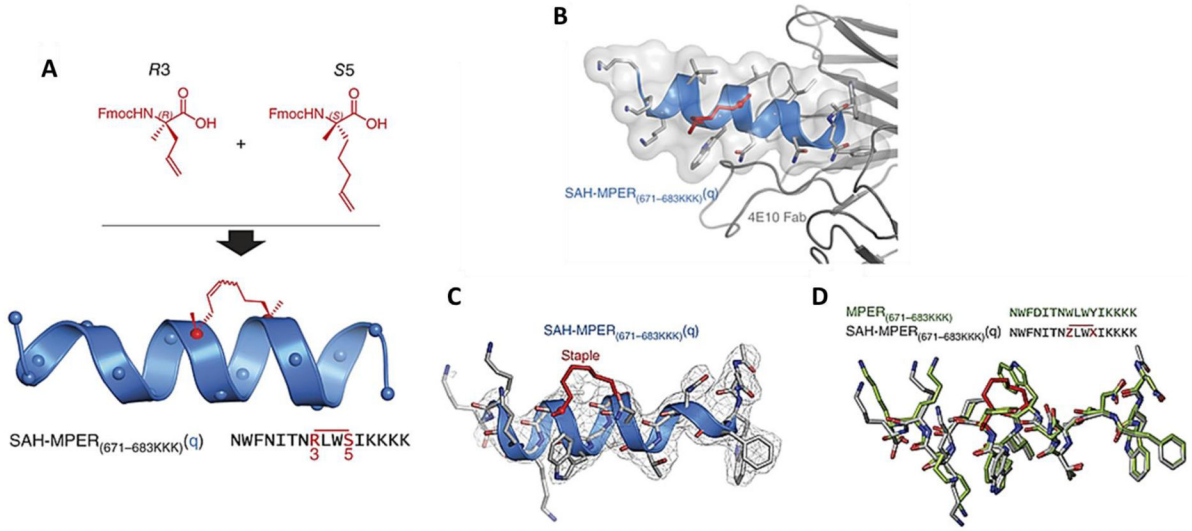

Fig. 19.

(A) Chemical optimization of i, i + 3 hydrocarbon stapling: Top, design and synthesis of SAH-MPER(671–683KKK)(q), in which the N-terminal S5 residue was replaced with R3 to lead to efficient i, i + 3 olefin metathesis under standard reaction conditions. (B) Crystal structure of SAH-MPER(671–683KKK)(q) (shown as a blue ribbon and gray transparent van der Waals surface) bound to 4E10 Fab, at 2.9-Å resolution. (C) 2Fo–Fc electron density map (1σ level) of the antibody-bound SAH-MPER(671–683KKK)(q) peptide. (D) Superposition of the native (green; PDB 2FX7)18 and i, i + 3–stapled (gray) MPER(671–683KKK) peptides, highlighting the similarity of antibody-bound structures, aside from the appended C-terminal lysines and the incorporated staple. Z and X represent R3 and S5, respectively, in the staple (red bar above sequence). Reprinted from [231].

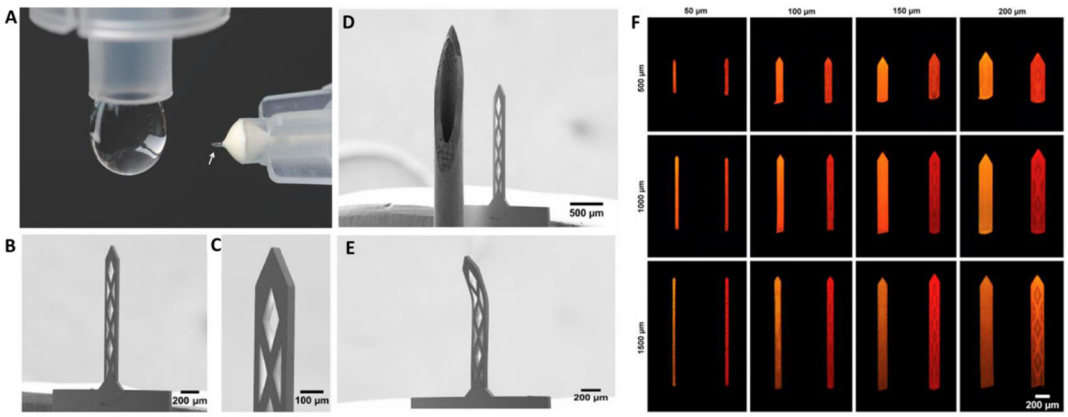

Fig. 20.

(A) Hollow microneedle (arrow), 720 μm in length, is shown next to a liquid drop of approximately 50-μL volume from a conventional eye dropper; Scanning electron micrographs of a representative fenestrated TiMN with 200 μm width, 50μm thickness, and 1500μm length: (B) Low magnification image showing the full needle, as well as a portion of its base; (C) Higher magnification image showing the needle tip and fenestrations; (D)Size comparison between a fenestrated TiMN and a conventional 26 gauge hypodermic needle typically used for intravitreal injection; (E) Buckled MN demonstrating graceful, plasticity-based failure mode after mechanical testing; (F) Fluorescence micrographs of PVP/Rhodamine B coated solid and fenestrated TiMNs. Reprinted from [253].

Table 1

List of FDA approved biopharmaceutical drugs

Drug	MW (kDa)	Route of administration	Half-life	FDA approval	Indication	Ref.
Adalimumab (Humira®)	148	Subcutaneous	~2 weeks (human)	July 2016	Uveitis	[15]
Aflibercept (Eylea®)	115	Intravitreal	3.63 days (rabbit); 7.1 days (human)	November 2011	Wet AMD	[16]
Ranibizumab (Lucentis®)	48	Intravitreal	2.88 days (rabbit); ~9 days (human)	June 2006, August 2012	Wet AMD, DME	[17]
Pegatanib sodium (Macugen®)	50	Intravitreal	~10 days (human)	December 2004	Wet AMD	[18]
Bevacizumab (Avastin®)	150	Intravitreal	4.32 days (rabbit); 4.9 days (human)	Off-label	Wet AMD	[19]

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Table 2

Proteins and peptides currently in clinical trials (Adapted and modified [41])

Protein/peptide drug	Description	MW (kDa)	Target/activity	Half-life	Company	Current indication	Phase	Ref.
AGN-150998 (Abiciparpegol)	Recombinant ankyrin repeat protein	34	VEGF-A	~2 weeks	Allergan	Wet AMD	Phase III	NCT02462486; NCT02462928
ALG-1001 (Luminate®)	Integrin peptide	1	Integrin receptors	~3 months	Allegro Ophthalmics	DME, NPDR	Phase II	NCT02348918
Conbercept (Lumitin®)	Recombinant fusion protein	143	VEGF-A/B, PGF-1	~1 week	Chengdu-Kanghong	Wet AMD, DME	Chinese FDA Phase III/ Phase II	NCT01809236
GSK933776	Anti-amyloid β antibody	NA	Amyloid β fibrils	~12 days	GlaxoSmithKline	Dry AMD	Phase II	NCT01342926
ISONEP™ (Sphingomab™)	Humanized antibody	~49	S1P	More than 4 days	Lpath	Wet AMD	Phase II	NCT01414153
Lampalizumab	Antigen-binding fragment of a humanized monoclonal antibody	47	CFD	~6 days	Roche	Geographic atrophy secondary to AMD	Phase III	NCT02247479
RN6G (PF-4382923)	Anti-amyloid β antibody	NA	Amyloid β fibrils	NA	Pfizer	Dry AMD	Phase II	NCT01003691
RTH258 ESBA1008	Antibody fragment	26	VEGF-A	~5 days	Novartis AG	Wet AMD	Phase III	NCT02507388
VGX-300 (OPT-302)	Recombinant fusion protein	NA	VEGF-C/D	NA	Circadian Opthea	Wet AMD	Phase I	NCT02543229
HI-con1	Recombinant fusion protein	NA	Tissue factor	NA	Iconic Therapeutics	AMD	Phase II	NCT02358889
Zimura	Aptamer	~50	Complement factor C5		Ophthotec Corporation	AMD, IPCV	Phase II/III	NCT02686658 NCT02397954

NPDR, nonproliferative diabetic retinopathy; S1P, sphingosine 1-phosphate; IPCV, Idiopathic polypoidal choroidal vasculopathy

Table 3

Permeability of proteins, peptides and macromolecules across ocular barriers

Compound	MW[a] (Da)	Tissue	Animal[b]	Permeability	Reference
Serum albumin	66000	cornea	H	5.48E-07	[51]
Inulin	5000	cornea	R	5.50E-07	[52]
Cyclosporine	1201	cornea	R	1.10E-05	[53]
Deoxycorticosterone	330	cornea	R	4.00E-05	[54]
Progesterone	314	cornea	R	2.00E-05	[54]
Testosterone	288	cornea	R	4.20E-05	[54]
Immunoglobulin	140000	stroma	R*	8.00E-09	[55]
Hemoglobin	64500	stroma	O	5.70E-07	[56]
Serum albumin	65000	stroma	R	1.40E-07	[57]
Dextran	75000	endothelium	R	7.50E-07	[58]
Serum albumin	65000	endothelium	R	8.30E-09	[55]
Poly(vinylpyrrolidone)	45000	endothelium	R	3.80E-07	[58]
Dextran	16000	endothelium	R	2.70E-05	[58]
Inulin	5000	endothelium	R	1.40E-06	[58]
Bevacizumab	145000	sclera	H	5.30E-07	[59]
Dextran-70	70000	sclera	H	1.90E-06	[60]
Serum albumin	65000	sclera	C	1.30E-07	[61]
Hemoglobin	64500	sclera	C	3.60E-07	[61]
Dextran-40	40000	sclera	H	4.30E-06	[60]
Dextran-10	10000	sclera	H	6.20E-06	[60]
Inulin	5000	sclera	C	1.90E-06	[61]
Inulin	5000	sclera	H	9.00E-06	[60]
Inulin	5000	sclera	R	2.50E-06	[52]
Hydrocortisone	362	sclera	C	6.50E-06	[61]
Inulin	5000	conjunctiva	R*	3.80E-06	[52]
FITC-dextran	77000	RPE-choroid	C	2.70E-08	[62]

[a] (MW) Molecular weight

[b] Source of tissue (R) rabbit, (C) cow, (O) ox, (H) human

All permeability measurements were obtained from in vitro experiments except those followed by an asterix (*), which were obtained from in vivo.

Table 4

Characteristics of various routes of administration for ocular drug delivery

ROUTES	NOTES
TOPICAL	
Drug entry pathways	Corneal, conjunctival, and scleral pathways.
Delivery barriers	Membrane barriers and elimination pathways on the eye surface, cornea, BRB, and tight junctions.
Elimination pathway	Tear wash out; nasolacrimal drainage; choroid, conjunctiva blood flow; lacrimation and blinking.
Advantages	High patient compliance; less systemic side effects; relatively easy and safe to administer.
Limitations	Small retention time of drug or dosage forms; blurring of vision; irritation; precorneal drug losses; drainage through the nasolacrimal duct; low bioavailability; limited volume of administration (approx. 30 μL); fast clearance from ocular surface; metabolism by tear enzymes; nonproductive uptake into systemic circulation via highly vascularized conjunctiva, choroid, uveal tract and inner retina; aqueous humor outflow gradient.
Approaches for improvement in therapeutic efficacy	Bioadhesive formulations may reduce precorneal clearance and increase corneal surface contact time. Positive charge of formulations may enhance the contact time with cornea to interact with negatively charged mucosa. Nanowafers approach may be beneficial for long-term and sustained drug release.
SYSTEMIC	
Drug entry pathways	Choroid and conjunctiva
Delivery barriers	Choroid and BRB (selectively permeable to highly lipophilic molecules).
Elimination pathway	Hepatic clearance; conjunctival and choroid capillaries and phagocytic clearance.
Advantages	Better patient compliance relative to intraocular injection.
Disadvantages	Low bioavailability due to the BRB, hence higher doses required which may produce systemic side effects.
Approaches for improvement in therapeutic efficacy	Large molecules and/or hydrophilic drugs are able to penetrate the choroid from the systemic circulation, but are unable to cross the inner BRB into the retina. Therefore, drugs must exit the choroidal circulation and permeate the outer BRB.
INTRAVITREAL	
Drug entry pathways	Directly to the vitreous chamber
Delivery barriers	Diffusion through the vitreous chamber, neural retina, and BRB.
Elimination pathway	Movement through aqueous chamber and retina; dynamic clearance mechanisms, such as anterior bulk aqueous flow or posterior vitreoretinal-choroidal flow, and elimination from the site of deposition.
Advantages	Local and direct delivery; high therapeutic concentration; no barrier to reach macula.
Disadvantages	It is necessary to administer the drug frequently to maintain adequate intraocular concentrations; frequent injections have been associated with adverse events especially retinal detachment, cataract, vitreous hemorrhage and endophthalmitis; linked to degeneration of PRs and cataracts and increase in IOP; only about 50–100 μl is administrable in human via intravitreal; high cost of administration of drugs (anti-VEGF).
Approaches for improvement in therapeutic efficacy	Extended drug release formulation for longer duration and/or drug modifications including specific properties such as size, charge, and lipophilicity; also need stimuli-responsive approach for drug release.
PERIOCULAR	
Drug entry pathways	Trans-scleral pathway to effectively deliver drugs next to the choroid.
Delivery barriers	Scleral thickness, choroidal blood circulation and BRBs.
Elimination pathway	Conjunctival and choroidal blood and lymphatic flow; losses from the periocular space, BRB, and choroidal circulation; drug binding to tissue proteins.
Advantages	Less invasive; high therapeutic drug levels; possible repetitive periocular administration under local anesthesia without direct interference with the vision. High volumes of drug solution can be administered in human and can bypass the BRB without intraocular penetration.

ROUTES	NOTES
Disadvantages	Rapid drug clearance; systemic side effects; tissue hemorrhage; and low retinal bioavailability compared to intravitreal injections; the injected drug still has to traverse the sclera, which is less permeable to larger molecules. The drugs have to pass through several layers including the episclera, sclera, choroid, BM, and RPE-while overcoming choroid circulatory clearance; the delivery is not as effective as intraocular injections in targeting retinal tissue.
Approaches for improvement in therapeutic efficacy	Improvements to formulations that either increase residence time or promote diffusion from the middle coat may be effective in overcoming the barriers to periocular delivery; nano-size formulations may provide superior diffusion; charge of formulations determines the interaction or diffusion process.
SUPRACHOROIDAL (SC)	
Drug entry pathways	Flow across the sclera is quick along the inner surface of the eye and subsequently into the posterior chamber.
Delivery barriers	Choroid and basement membrane.
Elimination pathway	High blood flow in the chorio-capillaries can wash away therapeutic molecules deposited in the SC space.
Advantages	Preferred site for drug delivery to the posterior tissues such as choroid, RPE and macula, due to its non-interference with the optical pathways and improved diffusional access to the choroid; this allows larger volumes of drugs with minimally invasive procedure; SC space can accommodate up to 1 ml of fluid, which rapidly diffuses into the posterior segment; injections of 10–50 μL into the SC space have been demonstrated to be well tolerated with lower risks for ocular complications.
Disadvantages	Injection of a drug solution into the SC space can result in rapid drug diffusion to cover the entire SC surface which may potentially induce drug-related toxicities of the surrounding tissues; rapid clearance of macromolecules occurs following suprachoroidal administration; postoperative inflammation and choroidal hemorrhage remain a concern and needs to be overcome while injecting into the SC space.
Approaches for improvement in therapeutic efficacy	Diffusion kinetics from the SC space could be optimized using sustained release formulations such as nano and microparticles; drug delivery systems that can provide controlled and continuous drug release are likely to minimize side-effects; such controlled devices might help overcome rapid fluctuation of the dosed drugs from conventional injectable solutions into the SC space and hence reduce toxicity to the surrounding tissues; MNs appear to offer a viable option for delivery of drugs to the back of the eye, especially when delivered through the SC route; these needles help to deposit drug or carrier system into sclera or into the SCS which may facilitate diffusion of drug into deeper ocular tissues, choroid and neural retina.

Table 5

A summary of advantages and limitations of various ocular delivery systems for protein and peptide based biopharmaceuticals

DELIVERY SYSTEMS	ADVANTAGES	LIMITATIONS
Micro and Nanoparticles	• Controlled and long-term drug release is possible using various routes of administration • Small size allows enhanced permeation into various organs • Greater flexibility for surface modification ligand molecules • High adjuvancy for vaccine • Encapsulation and delivery of various drugs on one nanocarrier • Adjustable physicochemical properties (size, shape, surface functionality) • Higher possibility of stimuli sensitive delivery • Targeted delivery system	• Burst release of drug can produce potential toxicity • Non-specific uptake by RES system and phagocytic clearance • Challenges include biocompatibility, toxicity, safety, stability, and immunotoxicity • Polymers have strong influence on drug release and stability • Various factors (size, shape, surface properties of carriers) affect release behavior, stability and targeting efficiency • Scale-up of nanoformulations development • Small size and large surface area may lead to particle aggregation • Non-uniformity of particle size distribution • Polymers hydrophobicity and acidic microenvironment created by polymer degradation leads to protein denaturation/aggregation • Chemical reactions between macromolecules and polymers
Liposomes	• Versatility of surface chemical modification and specific targeting • Entrapment of hydrophilic and hydrophobic drugs to aqueous and lipid phases, respectively • Can provide a sustained and controlled release • Drug release can be controlled, depending on the bilayer number and composition • Possibility of stimuli sensitive delivery system • Higher biocompatibility and non-immunogenicity	• Instability in biological media • Phagocytic uptake and clearance • Process of preparation of liposomes has stability issues on macromolecules • Manufacturing cost, scale up, batch-to-batch reproducibility • Production of sterile liposomes is expensive which reduces their applicability • Interactions of phospholipids with protein drugs • Heterogeneous particle size distribution
Solid lipid nanocarriers	• Large scale and effective production • Small size, large surface area and high drug loading • Improved drug stability • Avoidance of organic solvents in the preparation can minimize stability problems of macromolecules • Potential of carrying both lipophilic and hydrophilic drugs	• Complexity of the physical state of the lipid • Phagocytic uptake and clearance • Lipid particle growth, and tendency to gelation • Sometimes low drug loading capacity due to formation of lipid crystal matrix

DELIVERY SYSTEMS	ADVANTAGES	LIMITATIONS
	• Excellent biocompatibility	
Dendrimers	• Can be tailored by manipulating the structure/composition or number of surface functional groups • Thermodynamically stable system • Uniform size distribution • Drug molecules can be loaded both in the interior as well as attached to the surface groups • High transfection efficiency not only due to their well-defined shape, but may also be caused by the amine functionality	• Complexity of formulation methods • Toxicological issues are major limitations of dendrimers in clinical application • Core of structure is difficult to access as the complexity of the system increases with multiple generation structures
Hydrogels	• The porous nature of hydrogels can be finely tuned to allow better drug loading • Pharmacokinetic properties for release of the loaded therapeutic molecule can be easily adjusted to the requirements of an individual molecule • Biocompatible because of their high water content and soft nature • Unlike other delivery systems, organic solvents and protein denaturation processes are not used in hydrogel preparation procedures. This is beneficial in preserving protein stability, as very mild conditions (aqueous environment, room temperature) are generally used • Proteins have a limited mobility or are immobilized in the hydrogel network, which is favorable for preservation of their fragile 3D structure • Hydrogel's soft and hydrophilic nature and mild preparation methods are well-suited to improve efficacy, reduce dosing interval, and provide a more convenient dosage route for large and labile proteins • Stimuli sensitive hydrogel delivery is feasible	• High water content and soft nature of hydrogels typically results in relatively rapid release of proteins from the gel matrix • Burst release, low mechanical strength, and short durability • Protein damage due to encapsulation • Stability of hydrogels is poor in most cases and represents a major limitation • The low tensile strength of many hydrogels limits their use in load-bearing applications and can result in the premature dissolution or flow away of the hydrogel from a targeted local site • The quantity and homogeneity of drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs • Sometimes, hydrogels are not sufficiently deformable to be injectable, necessitating surgical implantation • Each of the above issues significantly restricts the practical use of hydrogel-based drug delivery therapies in the clinic
Micelles	• High diversity of polymers • Suitable for topical and intravitreal administration • Easy and reproducible formulation process • Ease of sterilization by simple filtration process for safe administration • High biocompatibility, biodegradability, and the multiplicity of functional groups • Possibilities of different polymer block arrangements based on the requirements • The hydrophobic core serves as a solubilization depot for drugs with poor aqueous solubility	• Toxicity and immunogenicity concern • Lack of suitable formulation methods for scale-up • Formulation instability • Low cellular uptake and tissue accumulation • Self-assembled polymeric micelles are not stable and may dissociate upon dilution. • Potential use in gene delivery is small and not well evaluated • Instability in the physiological environment • Micelles are liable to dissociate, especially upon administration when

DELIVERY SYSTEMS	ADVANTAGES	LIMITATIONS
	• The hydrophilic shell limits opsonin adsorption, which contributes towards a longer blood circulation time • The small size of polymeric micelles contributes towards longer blood circulation time by evading scavenging by the MPS system in the liver and bypassing the filtration of inter-endothelial cells in the spleen • Longer circulation time leads to improved accumulation at tissue sites with vascular abnormalities	they are diluted to a concentration below the CMC. • Limitations in entrapping hydrophilic small as well as macromolecule drugs.
Composite formulations (nanocarriers- in-gel)	• Minimizes the burst effect (dose dumping) of nanoformulations which may result in severe dose related toxicity • Exhibit nearly zero order release for longer period of time with no or minimal burst effect • This novel system provides stable environment for macromolecules against catalytic enzyme.	• Nanocarriers can be suspended in the gel at the time of delivery only otherwise drug will be released from the nanocarriers and accumulate in the gel which could give burst effect. Therefore, this novel approach requires dual chamber mixing device • Storage at cool temperature needed

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Table 6

Controlled-release systems being investigated for protein and peptide therapeutics for ocular implications (Adapted and modified from [41])

Formulation Approaches	Drug	Description	Release	Status	Ref.
Particulate systems					
Biodegradable polymeric microspheres					
PLGA MPs	Bevacizumab	Fabricated by Double emulsion method; particle size, 2–10 μm	62% released within 91 days	Preclinical, in vitro	[235]
PLA NPs within PLGA MPs	Bevacizumab	Fabricated by supercritical infusion and pressure quench technology	67% released within 120 days (4 months)	Preclinical, in vivo, rat model	[236]
PLGA MPs	Bevacizumab	Fabricated by solid-in-oil-in- hydrophilic oil method; particle size, 2–7 μm	NA	Preclinical, in vivo, rabbit model	[237]
Silicon dioxide MPs	Bevacizumab	Synthesized by electrochemical etching and oxidation of silicon wafer in hydrofluoric acid followed by ultrasonic fracture; particles with a pore size of 100 nm	165 days (5 months)	Preclinical, in vitro	[238]
Biodegradable polymeric nanospheres					
PLGA – albumin NPs	Bevacizumab	Fabricated by w/o/w double emulsion in presence of albumin as a stabilizer; particle size ~ 197 nm	165 days (5 months)	Preclinical, in vivo, rabbit model	[239], [240]
Liposome	Bevacizumab	EPC-Chol and DPC-chol liposomes formed by dehydration and rehydration method followed by freeze drying	NA	Preclinical, in vivo, rabbit model	[162]
Liposome–annexin	Bevacizumab	PC-PS-Chol-Toc liposomes fabricated by dehydration and rehydration method and subsequently coated with annexin; particle size, 100 nm	NA	Preclinical, in vivo, rabbit mode	[163]
Biodegradable and non-biodegradable polymeric implants					
ENV705 (intravitreal) Envisia therapeutics	Bevacizumab	Drug dispersed into biodegradable hydrogel-based matrix made by PRINT technology and molded into implants	2 months	Preclinical animal test	[241]
Nano-pores film device (intravitreal) Zordera Inc.	Ranibizumab	Biodegradable DDS based on PCL; drug pellet sandwiched between a nanopore and impermeable layer (total thickness 40 mm)	3 months	Under investigation	[241]
Posterior micropump system (subconjuctival)	Ranibizumab	Nonbiodegradable refillable DDS drug loaded with preprogrammed micropump; drug delivered in controlled nanodroplets	Long- term (refillable)	Phase I	[242]

Formulation Approaches	Drug	Description	Release	Status	Ref.
Port delivery system (PDS) (subconjuctival) Genentech	Ranibizumab	Semipermeable nonbiodegradable membrane with a refillable port; several exit ports to release drug into vitreous humor	Long- term – 1 year (refillable)	Phase II	[204]
Verisome IB20089 (intravitreal) Icon bioscience	Triamcinolo ne/ ranibizumab	Biodegradable with liquid gel or solid core; liquid injectable formulation; coalesces after intravitreal injections to form spherules	1 year	Phase II	[243]
In-situ gelling formulations					
Hyaluronic acid- dextran	Bevacizumab	Catalyst-free chemical crosslinking between vinylsulfone functionalized HA/ thiolated dextranin under physiological conditions; transparent gel formed in vitreous after injection; better sustained release observed in vivo compared with in vitro release	6 months	Preclinical, in vivo, rabbit model	[244]
Alginate-chitosan hydrogel/PLGA microspheres	Bevacizumab / ranibizumab	Antibody-loaded PLGA microspheres encapsulated into alginate hydrogels	196 days (6.5 weeks)	Preclinical, in vitro	[139]
Silk-based hydrogels	Bevacizumab	On the basis of physically crosslinked silk fibroin heavy chain (Mw = 350 kDa); biocompatible crosslinking reaction	Up to 3 months	Preclinical, in vitro	[216]
Diels–alder hydrogels	Bevacizumab	On the basis of PEG macro- monomers, chemically crosslinked by Diels–Alder reaction; mechanical stability enhanced	Up to 6 weeks	Preclinical, in vitro	[245]
Poly(2-ethyl-2- oxazoline)-b- poly(caprolactone)- b-poly(2-ethyl-2- oxazoline)	Bevacizumab	Reversible sol–gel transition; good in vitro and in vivo biocompatibility	20 days	Preclinical, in vitro	[213]
Poly(n- isopropylacrylamid e) pnipaam and poly(ethylene glycol diacrylate) peg-da	Bevacizumab / ranibizumab	Enhanced mechanical properties; good biocompatibility; thermoresponsive hydrogel; PNIPAAm shows LCST behavior	3 weeks	Preclinical, in vivo, rat model	[246]
PEG-poly- (serinolhexamethyl ene urethane)	Bevacizumab	Sol-to-gel phase transition when kept at in vivo temperatures; good in vitro and in vivo biocompatibility	17 weeks	Preclinical, in vivo, rabbit model	[217]
Delivery using living cells					
Renexus NT-501 (surgical) Neurotech Inc.	Cell line secreting CNTF	Nonbiodegradable; cell encapsulated in a semipermeable polysulfone capsule; Phase 2 results not encouraging because of adverse effects	18 months	Phase II/III	[247]
NT-503 (surgical) Neurotech Inc.	Cell line secreting VEGFR-Fc	Nonbiodegradable implant; semipermeable hollow fiber membrane encapsulating cells	12 months	Phase I	[248]

CNTF, ciliary neurotrophic factor; DDS, drug delivery systems; DPC-chol, 1,2 dipalmitoyl-sn-glycero-3-phosphocholine; EPC-chol, egg phosphatidylcholine-cholesterol; MPs, microparticles; NPs, nanoparticles; PLGA, polylactic-co-glycolic acid; PC-PS-Toc, egg phosphatidylcholine-porcine brain phosphatidylserine-tocopherol; PCL, polycaprolactone; PEG, polyethylene glycol; PLA, polylactic acid.