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Review Article

Nanotechnology-based drug delivery treatments and specific targeting therapy for age-related macular degeneration

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Abstract

Nanoparticles combined with cells, drugs, and specially designed genes provide improved therapeutic efficacy in studies and clinical setting, demonstrating a new era of treatment strategy, especially in retinal diseases. Nanotechnology-based drugs can provide an essential platform for sustaining, releasing and a specific targeting design to treat retinal diseases. Poly-lactic-co-glycolic acid is the most widely used biocompatible and biodegradable polymer approved by the Food and Drug Administration. Many studies have attempted to develop special devices for delivering small-molecule drugs, proteins, and other macromolecules consistently and slowly. In this article, we first review current progress in the treatment of age-related macular degeneration. Then, we discuss the function of vascular endothelial growth factor (VEGF) and the pharmacological effects of anti-VEGF-A antibodies and soluble or modified VEGF receptors. Lastly, we summarize the combination of anti-angiogenic therapy and nanomedicines, and review current potential targeting therapy in age-related macular degeneration.

Keywords: age-related macular degeneration; choroidal neovascularization; nanotechnology; target therapy; vascular endothelial growth factor

1. Introduction

Age-related macular degeneration (AMD) is a common degeneration of retina in aging people, characterized by a yellowish deposit in the macula in dry type and choroidal neovascularization (CNV) in wet type. Wet AMD, also named neovascular AMD, is the leading cause of visual impairment in the elderly in industrialized countries, and results in blurred central vision due to macular edema from vascular hyperpermeability and abnormal blood vessel growth behind the macula.[1] Vascular endothelial growth factor (VEGF), a protein essential in angiogenesis and vascular hyperpermeability, is highly associated with wet AMD. According to the severity of wet AMD, several treatment choices are available nowadays, including intravitreal injection of therapeutic agents, argon

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laser photocoagulation of abnormal vessels, photodynamic therapy with verteporfin, and vitreoretinal surgery as a final resolution. Argon laser photocoagulation has been used to directly destroy the abnormal CNV membrane since the 1970s,[2] playing an important role in the treatment of wet AMD before we knew the importance of VEGF. Photocoagulation is now used as a supplement to treat extrafoveal neovascularization and polypoidal choroidal vasculopathy, a variant of AMD, due to its tissue-destroying effect. Photodynamic therapy is targeted at the subfoveal CNV to prevent traditional laser burn in the macula.[3] It is performed with the assistance of an intravenously injected photosensitive agent, followed by exposure to light of a specific wavelength (689 nm). However, its clinical application is limited due to its high cost and possible choroidal ischemia after treatment.[4]

Considering the pathophysiology of wet AMD, it is believed that the causes of neovascularization are increased intraocular concentration of VEGF and macrophage-induced inflammation within retinal tissues. Thus, intravitreal injection of anti-VEGF agents and/or anti-inflammatory drugs (mainly steroids) monthly for at least three times has become a consensus in the treatment of patients with fresh and recurrent wet AMD.[5] Since intraocular delivery of a steroid may lead to elevated intraocular pressure or cataract formation, this treatment should be used selectively, especially in young patients.[6] In patients with refractory wet AMD presenting severe vitreous hemorrhage and epiretinal CNV membrane,[7] vitreoretinal surgery should be considered to remove the blood and restore the integrity of the retinal structure.

2. VEGF, anti-VEGF-A antibodies, and soluble or modified VEGF receptors

VEGF belongs to a growth factor family, which includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor.[8] VEGF-A induces hemangiogenesis through VEGF receptor-2 (VEGFR-2), and VEGF-C/VEGF-D stimulates lymphangiogenesis through VEGFR-2 and VEGFR-3.[9–12] Currently, anti-VEGF agents such as bevacizumab (Avastin, Genentech, CA, USA), ranibizumab (Lucentis, Genentech, CA, USA), and aflibercept (Eylea, Regeneron Pharmaceuticals, NY, USA) are available in the market. Bevacizumab is a whole anti-VEGF-A immunoglobulin, while ranibizumab is a fab fragment of an antibody against VEGF-A. By contrast, aflibercept is a new recombinant fusion protein that is composed of two main components, the VEGF binding portions from the extracellular domains of human VEGFR-1 and VEGFR-2, which is then fused to the Fc portion of human immunoglobulin G1.[13] Aflibercept has the ability to bind VEGF-A, VEGF-B, or placental growth factor and to inhibit angiogenesis. Currently, many studies are comparing the efficacy and safety of these three drugs.

By contrast, studies have found a common expression of soluble VEGFRs on the corneal surface. These truncated forms of VEGFR are formed by alternative splicing or proteolytic shedding, and can block the effect of VEGF ligand by VEGF trapping, further preventing their binding at membrane-bound VEGFRs. This mechanism helps inhibit angiogenesis and further maintains avascularity of cornea.[14–18] The soluble truncated form of VEGFR-1 (Flt-1), called fms-like tyrosine kinase (sFlt-1, sVEGFR-1), has high affinity for VEGF-A.[14] VEGFR-2 and VEGFR-3 both have their soluble forms, sVEGFR-2 and sVEGFR-3, respectively, and can block the function of VEGF-C.[16,18]

VEGFR intraceptor can also block VEGF. Lys-Asp-Glu-Leu (KDEL) is a quadripeptide retention signal that binds endoplasmic reticulum retention receptors. Thus, proteins coupled with KDEL cannot be secreted from the endoplasmic reticulum.[19] When domains 2 and 3 of VEGFR-1 are coupled to KDEL, the recombinant construct can bind VEGF intracellularly and block the function of VEGF. Flt23k (coupled domains 2–3 of Flt-1 with KDEL) was found to be a potential therapeutic agent for CNV in primate and murine AMD models.[20]

3. Nanotechnology-based drug delivery treatment

Most of the developing intraocular therapies are aiming at the reduction of macular edema or suppression of CNV resulting from elevated vitreous VEGF under several conditions, including not only AMD, but also diabetic retinopathy and retinal venous occlusion. Recently, naturally biodegradable or synthetic nanoparticulated drug delivery systems have been proposed as promising and alternative drug carriers in the treatment of retinal diseases. Nanotechnology can create and combine materials or devices with drugs and specially designed genes at a size of < 100 nm and, with advantages such as slow release, better tissue penetration, and higher drug packing, can help monitor, control, and cure diseases.[21] Fig. 1 shows the current applications of nanotechnology in ocular diseases, and the following section presents the details of current development of intraocular therapy with the use of nanomedicines.

3.1. Intraocular therapy by intravitreal injection

Current nanotechnology-based applications in intraocular therapy are summarized in Table 1 and described as follows.

3.2. VEGF-associated products

Nowadays, patients with neovascular AMD can reach better visual outcomes by the administration of different types of VEGF antagonists.[22] However, these treatments require frequent injections on a long-term basis, which may lead to patient noncompliance and increased risks of iatrogenic injury, such as bleeding, retinal detachment, and even endophthalmitis.[23] Therefore, a formulation with a stable, efficient, yet sustained releasing profile is necessary for the development of future therapeutics against AMD.

Poly-lactic acid (PLA) and poly-lactic-co-glycolic acid (PLGA) are biocompatible and biodegradable polymers, which have been approved by the Food and Drug Administration for use in drug products and widely studied for delivery

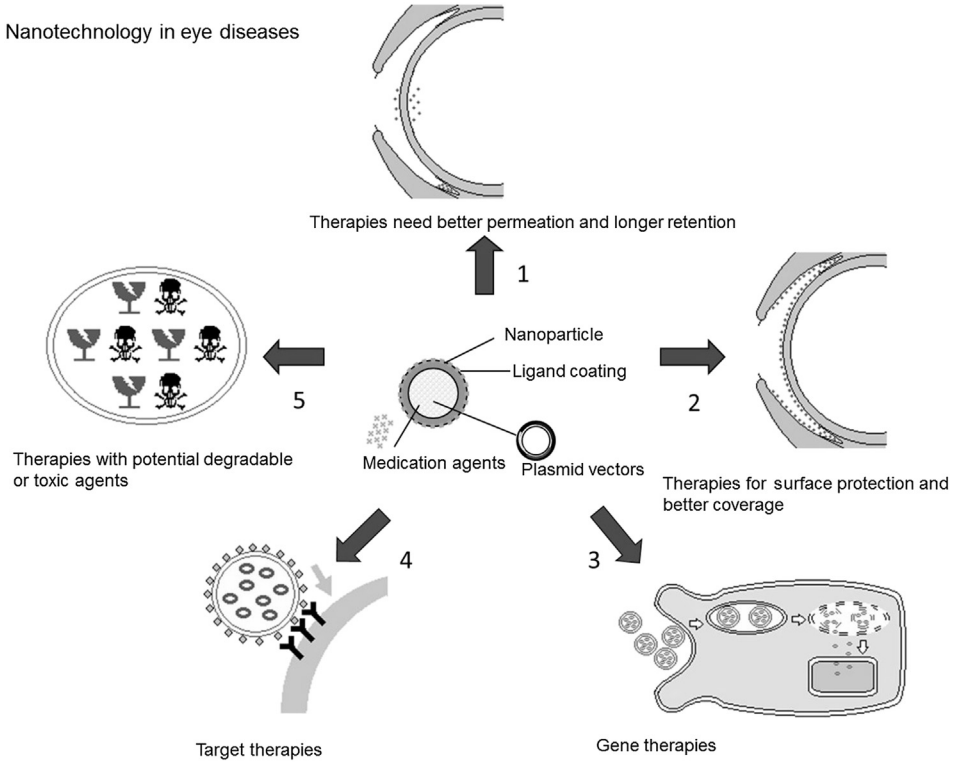

Fig. 1. Nanoparticles can contain medication agents and plasmid vectors, or have a ligand coating on their surface. Applications of nanotechnology in ocular diseases are divided into five parts: (1) nanotechnology-based drug delivery systems have better permeation and longer retention; (2) nanotechnology-based therapies can supply better surface protection and better coverage; (3) nanoparticles can be used in gene therapy; plasmid vectors in nanoparticles were phagocytosed by cells, and the genes were further released; (4) ligand-coated nanoparticles have a homing effect and can be applied for target therapies; and (5) nanotechnology-based therapies can prevent drugs from rapid degradation and further reduce drug toxicity.

of therapeutic molecules, especially proteins and peptides.[24] PLGA can be hydrolyzed to form natural metabolites (lactic and glycolic acids) and is eliminated from the body.[25] A number of approaches have been investigated for the preparation of drug-encapsulated polymers in the form of microparticles and nanoparticles.[26,27] The supercritical fluid technology is applicable for pharmaceutical manufacturing and generation of microparticle-based formulations for both small and large molecules.[28] While being above their critical point, supercritical fluids have fluid-like densities and gas-like diffusivity, which allow sufficient mixing under supercritical conditions. Yandrapu et al[29] prepared PLA nanoparticles in porous PLGA microparticles (NPinPMP) with bevacizumab using supercritical carbon dioxide. Supercritical carbon

Table 1
Current nanotechnology-based applications in ocular therapy

Group	APIs	Polymers	Techniques	Significant results
Yandrapu et al[29]	Bevacizumab	PLGA/PLA	Supercritical infusion and pressure quench technology	4 mo sustained release of bevacizumab (*in vitro*).2 mo release of bevacizumab in rat model (intravitreal administration)
Shmueli et al[36]	Serpin-derived peptide	Poly (beta-amino ester), PBAE, & PLGA	Self-assembling and microencapsulation	Zero-order *in vitro* release Significantly increased suppression of angiogenesis (>14 wk)
Luo et al[20]	Integrin-binding liner RGD peptide	PLGA	Emulsification solvent evaporation	Restored vision function after treatment with RGD-Flt23k-NR-nanoparticles in primate & murine macular degeneration models
Suen and Chau[35]	TA	Folate-functionalized poly(ethylene glycol)-b-polycaprolactone	Nanoprecipitation	Sustained TA release up to 4 wk at pH 5.5 & 8 wk at pH 7.4 Downregulation of VEGF and upregulation of pigment epithelium-derived factor lasting for at least 3 wk

APIs = active pharmaceutical ingredients; PBAE = poly (beta-amino ester); PLA = poly-lactic acid; PLGA = poly-lactic-co-glycolic acid; TA = triamcinolone acetonide; VEGF = vascular endothelial growth factor; RGD = Arg-Gly-Asp; NR= Nile Red; RGD-Flt23k-NR-nanoparticles = RGD-functionalized PLGA nanoparticles loaded with anti-angiogenic plasmid Flt23k and conjugated with Nile Red.

dioxide was able to expand the volume of PLGA microparticles by about sevenfold, but not that of the drug-coated PLA nanoparticles. The *in vitro* release of NPinPMP showed a sustained release of bevacizumab for 4 months. In addition, after intravitreal administration of NPinPMP in a rat model, bevacizumab was detectable for a prominently prolonged period (2 months), compared with the administration of bevacizumab solution alone (2 weeks).

Luo and colleagues[20] worked on targeted delivery of integrin-binding linear RGD (Arg-Gly-Asp) peptide against VEGF using PLGA nanoparticles with the aid of recombinant Flt23k intraceptor plasmid, which is capable of homing the delivery system to neovascular lesions in the retina and regressing CNV in both primate and murine AMD models. The delivery system showed no ocular and systemic toxicity. After intravenous injection, RGD-functionalized nanoparticles were shown to be localized at CNV lesions. More excitingly, restoration of vision function after treatment with RGD-Flt23k nanoparticles was validated in both primate and murine AMD models. Luo et al's[20] results are important in that RGD-targeted nanoparticles delivering Flt23k intraceptor plasmids provide a different approach for ocular orientation through systemic administration (intravenous injection). This treatment can abolish neovascularization and fibrosis in animal AMD models while preventing serious side effects related to intraocular injection of anti-VEGF agents.

3.3. Steroids

To reduce the side effects of intravitreal injection of steroids, Daull and colleagues[30] used a lipophilic prodrug of dexamethasone (DXM), dexamethasone palmitate (DXP), for treatment of macular edema in several animal models, including rats, rabbits, cats, and minipigs. They tried to characterize the sustained release capacity, efficacy, and safety of intravitreal injection of DXP emulsions in preclinical situations. The results showed good efficacy in both rat and rabbit models. In addition, the pharmacokinetic data of rabbit presented sustained DXM concentrations in the retina and choroid. The DXM concentrations were found to be sufficient to suppress VEGF-induced vascular hyperpermeability up to 9 months. Therefore, intravitreal injection of DXP emulsion was efficacious for a sustained drug release, and potentiated the control of vascular leakage for up to 9 months following only a single dose of intravitreal injection. Daull et al's[30] study demonstrated that intravitreal injection of DXP emulsion could be a safe and effective alternative drug delivery platform for corticosteroids against macular edema.

Ozurdex, an intravitreal DXM implant (Allergan Inc., Irvine, CA, USA), has been approved for the treatment of macular edema in branch retinal venous occlusion and central retinal venous occlusion.[31,32] However, it is also regarded as an adjunct therapy for patients with wet AMD refractory to anti-VEGF agents. Calvo et al[33] found Ozurdex to be effective in vision stabilization, reduction of intra- and subretinal fluids, and improvement of central retinal thickness in eyes with refractory wet AMD. It is a slow-releasing DXM implant that is injected through the pars plana. This implant is composed of a biodegradable, PLGA-based (NOVADUR solid polymer delivery system) copolymer matrix, which helps control the release of DXM, and allows DXM to maintain a high concentration in the vitreous during the first 2 months after injection and at a lower concentration later for up to 6 months.[32,34]

Recently, Suen and Chau[35] developed triamcinolone acetonide (TA)-loaded, folate-modified poly (ethylene glycol)-b-polycaprolactone (folate-PEG-b-PCL) nanoparticles by nanoprecipitation. A sustained release of TA from the nanoparticles was achieved for up to 4 weeks at pH 5.5 and 8 weeks at pH 7.4. In the cell (ARPE-19 cells) uptake experiment, down-regulation of VEGF and upregulation of pigment epithelium-derived factor, lasted for at least 3 weeks. This nanoparticle-based TA delivery system was highlighted as a possible replacement for the current intravitreal injection treatment. Furthermore, folate modification enhances the solubility of TA, while the encapsulation of TA reduces its possible cytotoxicity.

3.4. Other materials with antiangiogenic agents

Shmueli et al[36] discovered several classes of peptides with very strong antiangiogenic properties. In order to prevent peptide degradation, serpin-derived peptide was first self-assembled with positively charged poly (beta-amino ester), to form the nanoparticles. To further prolong the release, the manufactured polymer–peptide nanoparticles were then encapsulated into PLGA microparticles. The peptide-carrying microparticles showed nearly zero-order release of the peptide for up to 200 days. In addition, long-term *in vivo* studies in the laser-induced neovascularization rat model showed that these peptide-releasing microparticles suppressed angiogenesis for $>$ 14 weeks *in vivo* after a single intravitreal injection, revealing that the combination of serpin-derived peptide and biodegradable polymer-based particles was likely to be a suitable tool for future long-term treatment of chronic ocular diseases, especially AMD.

Celecoxib is a cyclooxygenase-2 inhibitor, characterized by its anti-inflammatory, antiproliferative, and anti-VEGF effects on the retina.[37] Palamoor and Jablonski[38] prepared celecoxib-loaded poly (ortho ester) nanoparticles for intraocular drug delivery against AMD and diabetic retinopathy, the two leading causes of blindness in the elder. Their nanoparticulated formulation showed a zero-order release due to surface erosion and no burst release of celecoxib. Moreover, in the cell-uptake study, the formulated nanoparticles were not internalized by Müller and HEK 293 cells, which implied that these nanoparticles could be a suitable platform for drug delivery specific to the posterior segment of the eyes against AMD.

3.5. Intraocular therapies by other routes

Although intraocular therapy with intravitreal injection directly delivers drug compounds to the posterior segment of the eye, potential side effects limit long-term therapy.[23] However, achieving therapeutic concentrations of drugs by

conventional oral and intravenous routes is difficult because of the presence of ocular barriers.[39] Drug administration by subconjunctival route is less invasive and considered as an alternative to the intravitreal route, but such treatments require frequent intervention.[40] Subconjunctival administration of drugs with nanoparticles can be further dispersed in PLGA–PEG–PLGA thermosensitive gels to sustain the drug release.[39,41] After injection, these thermogelling systems form a depot above the sclera and slowly release drugs for the treatment of diseases in the posterior segment. Boddu et al[39] prepared nanoparticulated gel formulations of steroids by oil-in-water emulsion and dialysis methods using PLGA 50:50 and PLGA 65:35. They found that the nanoparticles suspended in thermosensitive gels released steroids obeying zero-order kinetics without an apparent burst effect, and concluded that these novel systems could provide sustained retinal/choroidal delivery of a steroid following episcleral administration. Further studies are needed to evaluate the effects of other medications, such as anti-VEGF agents, in these thermosensitive nanoparticulate gel systems for creating a safer way to treat CNV in AMD.

4. Specific targeting therapy for AMD

4.1. Complement-associated specific targeting therapy

Since 2005, several studies have found that AMD is associated with a variation in the gene that encodes complement factor H (CFH), and in patients who have one copy of Y402H polymorphism, there is a nearly fivefold increase in the risk of having AMD.[42–45] It implies that dysregulation of the complement system has a role in the pathogenesis of AMD. The most well-known CFH genetic variant of AMD is the Y402H polymorphism (a tyrosine-to-histidine substitution at amino acid position 402 within the CFH protein). Besides CFH, other complement pathways encoding genes such as complement factor B, C2, C3, and I were also reported to be associated with the risk of AMD.[46] Therefore, molecular targeting of the complement pathways may help in the prevention and treatment of AMD. Currently, clinical trials of Potentia (C3 inhibitor), ARC1905 (C5 inhibitor), eculizumab (humanized monoclonal antibodies binding C5), Tanox (complement factor D inhibitor), and TA106 (complement factor B inhibitor) are in process.[47] C5a receptor antagonists, JSM-7717 and JPE-1375, are also currently in preclinical assessment.[47]

Regarding CFH, Wyatt et al[48] used quantitative yeast two-hybrid and enzyme-linked immunosorbent assays with different recombinant protein constructs, finding higher affinity of Fib3 for the disease-related CFH 402H variant. In two AMD tissue donors who were homozygous for CFH 402H (H/H), colocalization of CFH and Fib3 in globular deposits within soft drusen was also observed, which was distinct from those seen in eyes with Y/Y and H/Y genotypes. It was concluded that CFH 402H/Fib3 interaction could aggregate the development of soft drusen and could be a target for therapeutic intervention.

4.2. Interleukin-17

AMD is thought to be associated with the inflammatory response in the macula, where several inflammatory signals are involved, such as interleukin (IL). The IL-17 cytokine family contained six members named A–F, among which IL-17A is the main cytokine. IL-17A and IL-17 receptor (R)-C increase in AMD eyes and blood, implying IL-17 involvement in AMD pathogenesis. Targeting IL-17, IL-17R-C, or cells producing IL-17 may lessen retinal degeneration and could be considered as a potential therapeutic target for AMD.[49]

4.3. Apurinic endonuclease 1/redox factor-1

Inhibition of the redox function of apurinic endonuclease 1/redox factor-1 (APE1/Ref-1) can suppress endothelial angiogenesis and promote neuronal cell recovery, and is regarded as a potential treatment for AMD. Li et al[50] found that a small-molecule compound E3330, a specific inhibitor of APE1 redox function, can regulate retinal pigment epithelium (RPE) cell response to oxidative stress. E3330 can block sublethal doses of oxidized low-density lipoprotein, decrease the proliferation decline and aging of RPEs, and lessen intracellular reactive oxygen species, monocyte chemoattractant protein-1, VEGF, and nuclear factor-κB p65 in RPEs. An animal study also demonstrated that intravitreal injection of E3330 could effectively treat laser-induced CNV in mouse eyes.

4.4. Rap1 GTPase

Dysfunction of barrier of RPE contributes to one of the mechanisms of development of AMD. Regulation of endothelial and epithelial cell junctions is associated with Rap1 GTPase, which has two isoforms, Rap1A and Rap1B. Wittchen et al[51] used shRNA of Rap1 isoforms in cultured human RPE cells and knockout mouse models to test the role of Rap1 in RPE cells. They found that Rap1A and Rap1B are involved in steady-state barrier integrity and dynamic junctional responses of RPE cells, respectively. In the laser injury model, Rap1b(−/−) mice had larger CNV volumes, compared to wild-type or Rap1a(−/−) mice. Intravitreal injection of a Rap1 activator, a cAMP analog (8CPT-2′-O-Me-cAMP), could significantly reduce the CNV volume. They concluded that 8CPT-2′-O-Me-cAMP might be a potential treatment option for AMD.

In conclusion, to date, anti-VEGF drugs are the most widely used medications for neovascular AMD. However, there are still limitations in the clinical use of these drugs. Additionally, there is still no promising treatment for dry AMD. In recent years, nanotechnology-based drug delivery treatments for intraocular therapies of AMD have attracted a great deal of interests. PLA- and PLGA-based drug delivery systems are under investigation. We have summarized the recent developments of nanotechnology-based drug delivery treatments and specific targeting therapy of AMD in this article. We hope that one day these advancements will have

great outcomes in treating this common and destructive retinal disorder.

References

1. Congdon N, O'Colmain B, Klaver CC, Klein R, Munoz B, Friedman DS, et al. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol* 2004;**122**:477−85.
2. Macular Photocoagulation Study Group. Laser photocoagulation of subfoveal recurrent neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial. *Arch Ophthalmol* 1991;**109**:1232−41.
3. Kaiser PK, Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: 5-year results of two randomized clinical trials with an open-label extension—TAP report no. 8. *Graefes Arch Clin Exp Ophthalmol* 2006;**244**:1132−42.
4. Veritti D, Lanzetta P. Triple therapy for anti-vascular endothelial growth factor nonresponders in neovascular age-related macular degeneration: impact of different photodynamic therapy parameters. *Ophthalmologica* 2013;**230**:131−7.
5. Peyman GA, Lad EM, Moshfeghi DM. Intravitreal injection of therapeutic agents. *Retina* 2009;**29**:875−912.
6. Jonas JB. Intravitreal triamcinolone acetonide for treatment of intraocular oedematous and neovascular diseases. *Acta Ophthalmol Scand* 2005;**83**:645−63.
7. Jung JH, Lee JK, Lee JE, Oum BS. Results of vitrectomy for breakthrough vitreous hemorrhage associated with age-related macular degeneration and polypoidal choroidal vasculopathy. *Retina* 2010;**30**:865−73.
8. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 1999;**13**:9−22.
9. Goldman J, Rutkowski JM, Shields JD, Pasquier MC, Cui Y, Schmokel HG, et al. Cooperative and redundant roles of VEGFR-2 and VEGFR-3 signaling in adult lymphangiogenesis. *FASEB J* 2007;**21**:1003−12.
10. Achen MG, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks AF, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci U S A* 1998;**95**:548−53.
11. Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J* 1996;**15**:290−8.
12. Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer* 2011;**2**:1097−105.
13. Papadopoulos N, Martin J, Ruan Q, Rafique A, Rosconi MP, Shi E, et al. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF trap, ranibizumab and bevacizumab. *Angiogenesis* 2012;**15**:171−85.
14. Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T, et al. Corneal avascularity is due to soluble VEGF receptor-1. *Nature* 2006;**443**:993−7.
15. Ambati BK, Patterson E, Jani P, Jenkins C, Higgins E, Singh N, et al. Soluble vascular endothelial growth factor receptor-1 contributes to the corneal antiangiogenic barrier. *Br J Ophthalmol* 2007;**91**:505−8.
16. Albuquerque RJ, Hayashi T, Cho WG, Kleinman ME, Dridi S, Takeda A, et al. Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. *Nat Med* 2009;**15**:1023−30.
17. Pavlakovic H, Becker J, Albuquerque R, Wilting J, Ambati J. Soluble VEGFR-2: an antilymphangiogenic variant of VEGF receptors. *Ann N Y Acad Sci* 2010;**1207**(Suppl. 1):E7−15.
18. Singh N, Tiem M, Watkins R, Cho YK, Wang Y, Olsen T, et al. Soluble vascular endothelial growth factor receptor 3 is essential for corneal alymphaticity. *Blood* 2013;**121**:4242−9.
19. Jani PD, Singh N, Jenkins C, Raghava S, Mo Y, Amin S, et al. Nanoparticles sustain expression of Flt intraceptors in the cornea and inhibit injury-induced corneal angiogenesis. *Invest Ophthalmol Vis Sci* 2007;**48**:2030−6.
20. Luo L, Zhang X, Hirano Y, Tyagi P, Barabas P, Uehara H, et al. Targeted intraceptor nanoparticle therapy reduces angiogenesis and fibrosis in primate and murine macular degeneration. *ACS Nano* 2013;**7**:3264−75.
21. Zarbin MA, Montemagno C, Leary JF, Ritch R. Nanotechnology in ophthalmology. *Can J Ophthalmol* 2010;**45**:457−76.
22. Frampton JE. Ranibizumab: a review of its use in the treatment of neovascular age-related macular degeneration. *Drugs Aging* 2013;**30**:331−58.
23. van Wijngaarden P, Coster DJ, Williams KA. Inhibitors of ocular neovascularization: promises and potential problems. *JAMA* 2005;**293**:1509−13.
24. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers (Basel)* 2011;**3**:1377−97.
25. Giordano GG, Chevez-Barrios P, Refojo MF, Garcia CA. Biodegradation and tissue reaction to intravitreous biodegradable poly(D,L-lactic-co-glycolic) acid microspheres. *Curr Eye Res* 1995;**14**:761−8.
26. Kompella UB, Bandi N, Ayalasomayajula SP. Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Invest Ophthalmol Vis Sci* 2003;**44**:1192−201.
27. Ungaro F, d'Angelo I, Miro A, La Rotonda MI, Quaglia F. Engineered PLGA nano- and micro-carriers for pulmonary delivery: challenges and promises. *J Pharm Pharmacol* 2012;**64**:1217−35.
28. Zarena AS, Sankar KU. Design of submicron and nanoparticle delivery systems using supercritical carbon dioxide-mediated processes: an overview. *Ther Deliv* 2011;**2**:259−77.
29. Yandrapu SK, Upadhyay AK, Petrash JM, Kompella UB. Nanoparticles in porous microparticles prepared by supercritical infusion and pressure quench technology for sustained delivery of bevacizumab. *Mol Pharm* 2013;**10**:4676−86.
30. Daull P, Paterson CA, Kuppermann BD, Garrigue JS. A preliminary evaluation of dexamethasone palmitate emulsion: a novel intravitreal sustained delivery of corticosteroid for treatment of macular edema. *J Ocul Pharmacol Ther* 2013;**29**:258−69.
31. Haller JA, Bandello F, Belfort Jr R, Blumenkranz MS, Gillies M, Heier J, et al. Randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with macular edema due to retinal vein occlusion. *Ophthalmology* 2010;**117**. 1134−46.e3.
32. Haller JA, Bandello F, Belfort Jr R, Blumenkranz MS, Gillies M, Heier J, et al. Dexamethasone intravitreal implant in patients with macular edema related to branch or central retinal vein occlusion twelve-month study results. *Ophthalmology* 2011;**118**:2453−60.
33. Calvo P, Ferreras A, Al Adel F, Wang Y, Brent MH. Dexamethasone intravitreal implant as adjunct therapy for patients with wet age-related macular degeneration with incomplete response to ranibizumab. *Br J Ophthalmol* 2015;**99**:723−6.
34. Chang-Lin JE, Attar M, Acheampong AA, Robinson MR, Whitcup SM, Kuppermann BD, et al. Pharmacokinetics and pharmacodynamics of a sustained-release dexamethasone intravitreal implant. *Invest Ophthalmol Vis Sci* 2011;**52**:80−6.
35. Suen WL, Chau Y. Specific uptake of folate-decorated triamcinolone-encapsulating nanoparticles by retinal pigment epithelium cells enhances and prolongs antiangiogenic activity. *J Control Release* 2013;**167**:21−8.
36. Shmueli RB, Ohnaka M, Miki A, Pandey NB, Lima e Silva R, Koskimaki JE, et al. Long-term suppression of ocular neovascularization by intraocular injection of biodegradable polymeric particles containing a serpin-derived peptide. *Biomaterials* 2013;**34**:7544−51.
37. Amrite AC, Kompella UB. Celecoxib inhibits proliferation of retinal pigment epithelial and choroid-retinal endothelial cells by a cyclooxygenase-2-independent mechanism. *J Pharmacol Exp Ther* 2008;**324**:749−58.
38. Palamoor M, Jablonski MM. Synthesis, characterization and *in vitro* studies of celecoxib-loaded poly(ortho ester) nanoparticles targeted for intraocular drug delivery. *Colloids Surf B Biointerfaces* 2013;**112**:474−82.

39. Boddu SH, Jwala J, Vaishya R, Earla R, Karla PK, Pal D, et al. Novel nanoparticulate gel formulations of steroids for the treatment of macular edema. *J Ocul Pharmacol Ther* 2010;**26**:37−48.
40. Geroski DH, Edelhauser HF. Drug delivery for posterior segment eye disease. *Invest Ophthalmol Vis Sci* 2000;**41**:961−4.
41. Amrite AC, Kompella UB. Size-dependent disposition of nanoparticles and microparticles following subconjunctival administration. *J Pharm Pharmacol* 2005;**57**:1555−63.
42. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;**308**:419−21.
43. Edwards AO, Ritter 3rd R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science* 2005;**308**:421−4.
44. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, et al. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;**308**:385−9.
45. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* 2005;**102**:7227−32.
46. Francis PJ, Klein ML. Update on the role of genetics in the onset of age-related macular degeneration. *Clin Ophthalmol* 2011;**5**:1127−33.
47. Troutbeck R, Al-Qureshi S, Guymer RH. Therapeutic targeting of the complement system in age-related macular degeneration: a review. *Clin Exp Ophthalmol* 2012;**40**:18−26.
48. Wyatt MK, Tsai JY, Mishra S, Campos M, Jaworski C, Fariss RN, et al. Interaction of complement factor h and fibulin3 in age-related macular degeneration. *PLoS One* 2013;**8**:e68088.
49. Chan CC, Ardeljan D. Molecular pathology of macrophages and interleukin-17 in age-related macular degeneration. *Adv Exp Med Biol* 2014;**801**:193−8.
50. Li Y, Liu X, Zhou T, Kelley MR, Edwards P, Gao H, et al. Inhibition of APE1/Ref-1 redox activity rescues human retinal pigment epithelial cells from oxidative stress and reduces choroidal neovascularization. *Redox Biol* 2014;**2**:485−94.
51. Wittchen ES, Nishimura E, McCloskey M, Wang H, Quilliam LA, Chrzanowska-Wodnicka M, et al. Rap1 GTPase activation and barrier enhancement in RPE inhibits choroidal neovascularization *in vivo*. *PLoS One* 2013;**8**:e73070.