Advanced DDS for Delivering Anti-VEGF Agents

Subjects: Ophthalmology

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The treatment of posterior segment eye diseases is challenging due to the complex anatomy of the eye, which limits the effective delivery of medications. Conventional treatments such as topical eye drops and intravitreal injections have poor bioavailability and short residence time, requiring frequent dosing. Biodegradable nano-based drug delivery systems (DDSs) offer a potential solution to these limitations, with longer residence time in ocular tissues and better penetration through ocular barriers. These DDSs use biodegradable polymers that are nanosized, reducing the risk of toxicity and adverse reactions.

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1. Anti-VEGF Agents

Pathological neovascularization is involved in various retinal diseases, including proliferative diabetic retinopathy (PDR), retinopathy of prematurity (RoP), and retinal vein occlusion (RVO), caused by retinal hypoxia [1][2]. On the other hand, choroidal neovascularization (CNV) is often caused by a ruptured or damaged Bruch's membrane and is associated with various retinal disorders, such as wet age-related macular degeneration, pathologic myopia, presumed ocular histoplasmosis syndrome (POHS), and traumatic choroidal rupture [3]. Anti-VEGF agents are the gold standard treatment for ocular neovascular diseases, including PDR, RoP, RVO, and CNV. Bevacizumab (Avastin™), Ranibizumab (Lucentis™), Aflibercept (Eylea™), and Pegaptanib (Macugen®) are commonly used anti-VEGF drugs [4]. These monoclonal antibodies target retinal and choroidal endothelial cells to stop angiogenesis. However, their large size limits their penetration through ocular barriers, resulting in poor bioavailability and the need for frequent intravitreal injections [4].

Non-degradable implants have been suggested as an alternative for anti-VEGF delivery. However, they have some drawbacks. Although they provide long-term drug release, their removal requires secondary surgery, which is associated with additional risks and potential complications. Additionally, these implants can cause several issues, such as impacting the visual axis due to their large size and migration to the anterior chamber, leading to corneal

edema and permanent endothelial decompensation caused by direct contact with the endothelium, mechanical trauma, or chemical toxicity [5].

The use of biodegradable nanocarriers for sustained-release of anti-VEGF drugs presents a potentially favorable option for enhancing the efficacy, bioavailability, bioactivity, duration of action, and safety of treatment, while minimizing the adverse effects associated with non-degradable implants. These carriers utilize biopolymers that can be gradually degraded within the eye, eliminating the need for a second surgery, and their release rate can be adjusted by modifying the composition and molecular weight of the carrier. Additionally, the small size of these carriers ensures optical clarity and reduces the risk of visual disturbances.

The most commonly used anti-VEGF drugs in ocular drug delivery systems are bevacizumab, aflibercept, and ranibizumab [6]. Bevacizumab is frequently used in studies and for wet-AMD due to its well-known toxicity and pharmacokinetic characteristics [7]. Aflibercept and ranibizumab are also used for treating wet-AMD. Aflibercept is a potent drug that requires less frequent dosing and may improve patient adherence, but there are concerns about rare but severe adverse effects [8]. It is also more expensive than bevacizumab, which may explain why it is not commonly used in current clinical practice.

2. Novel DDS for Anti-VEGF Agents

Nanocarriers, which can be lipid-based, polymers, or inorganic nanoparticles, present advantages and challenges, and can be classified according to their material components. However, protecting anti-VEGF agents from conformational changes and preserving their bioactivity while minimizing interaction with the nanocarrier is still a challenge, as too strong interactions can compromise drug capture and release processes and lead to protein denaturation [9].

2.1. Hydrogel

Hydrogel nanocarriers are a promising option for drug delivery due to their unique properties. They are three-dimensional polymer networks with a porous structure that can interact with water-soluble molecules. Despite a high initial burst of drug release, they are biocompatible, biodegradable, and safe. Hydrogel nanocarriers can carry water molecules while remaining in a solid state, and are known to maintain drug stability. However, their delicate structure can be affected by sterilization processes.

Hydrogels that can change their properties in response to environmental triggers, such as pH or temperature changes, are often referred to as “smart” hydrogels [10]. In a study by Osswald et al., a thermoresponsive hydrogel composed of suspended PLGA microspheres was developed to carry ranibizumab and aflibercept [11]. The addition of microspheres in the hydrogel resulted in extended drug release by 27.2%, with the DDS remaining bioactive for 196 days in vitro. The DDS showed promising results in inhibiting human umbilical vein endothelial cell (HUVEC) proliferation, which prompted further experiments on in vivo models. In a subsequent study on laser-induced rat CNV models, the nanotherapeutic significantly reduced CNV lesion areas by 60% compared to the control group in

vivo [12]. Over the course of 12-week treatment, less drugs were needed in the novel nanotherapeutics compared to the standard posology delivered via bolus administration. However, it is important to note that the small animal samples per treatment group, which was of four eyes, limited the results. The potential advantages of this DDS over standard treatment include limiting toxicity related to high drug dosage.

Hu et al. developed a thermoresponsive mPEG-PLGA-BOX hydrogel to test the efficacy of bevacizumab in inhibiting angiogenesis induced by retinal laser photocoagulation [13]. The hydrogel transitioned from a solution phase to a gel-phase after body temperature exposure, and both in vitro and in Rex rabbits, it successfully inhibited angiogenesis for 35 days while maintaining the anti-angiogenic bioactivity of bevacizumab. No cytotoxic effects were reported during nanocarrier biodegradation in the rabbits. Although this study was conducted on only 11 Rex rabbits divided into two groups, the results are promising, demonstrating the potential of DDS as a novel therapeutic gelling carrier against angiogenesis.

Xue et al. developed a thermoresponsive hydrogel made of PED–PPG–PCL and encapsulated bevacizumab and aflibercept [14]. In vitro tests on HUVEC showed that both drugs significantly inhibited proliferation. Ex vivo choroidal sprouting model studies also showed that both drugs, when independently injected with the nanocarrier, significantly reduced the relative sprouting percentage by more than 80%. Anti-angiogenic effects were observed ex vivo and in vivo in a persistent retinal neovascularization rabbit model. The drug release rate was extended by fine-tuning the hydrophilic/lipophilic ratio of the hydrogel, with the longest drug release of 40 days in vitro and at least 28 days in vivo achieved by increasing the hydrogel concentration to 20 weight percent with an optimized PEG/PPG ratio of 4:1. This DDS has a prolonged drug release rate that can be extended via polymer fine-tuning and represents a promising bioactive drug carrier.

Studies on the use of thermoresponsive hydrogels as drug delivery systems (DDS) have yielded optimistic results in vitro and in vivo, although for limited time periods. For instance, Liu et al. investigated the use of a thermoresponsive hydrogel to deliver bevacizumab over six months in vitro using PGLA in a PEG–PLLA–DA/NIPAAm hydrogel loaded with ranibizumab [15]. By optimizing the cross-linker concentration and microsphere load quantity, the hydrogel achieved enhanced biodegradability, drug release, and needle-injection feasibility. The hydrogel was effective in vitro for 190 days and was also tested with aflibercept in vitro, which was successfully released for six months while maintaining bioactive therapeutic levels [16]. The biodegradable cross-linker PEG–PLLA–DA prolonged the hydrogel nanocarrier degradation. Liu et al. further evaluated the nanotherapeutic's efficacy by intravitreally injecting it into a laser-induced CNV rat model, where it proved as effective as bimonthly aflibercept injections for six consecutive months, while avoiding inflammation and ocular complications [17]. This nanotherapeutic showed promising results on the rodent eye model, but its applicability to humans may be limited by anatomical differences and potential differences in drug pharmacokinetics and immune reactions.

Fan et al. demonstrated a promising approach for delivering conbercept, a novel anti-VEGF drug with a short half-life, using a short chain pH-sensitive peptide hydrogel DDS in vitro [18]. The nano-based DDS inhibited HREC proliferation and tube formation, indicating its potential therapeutic application for neovascular AMD. Moreover, the

hydrogel peptide nanocarrier demonstrated good biocompatibility with HRECs. However, further in vivo studies are necessary to determine the pharmacokinetics of the DDS, and therefore, the results remain preliminary.

Li et al. developed an injectable hydrogel (cSA@Lip-HAC) loaded with sunitinib and acriflavine liposomes, which demonstrated high antiangiogenic properties in vitro and increased drug residency in vivo [19]. The combination of co-drug-loaded liposomes in the hydrogel and subtenon administration route led to increased efficacy and significant anti-CNV results. The DDS remained active for 21 days in vivo, showing promise for a novel therapeutic avenue with fewer complications than with the intravitreal route. However, the limited time of the DDS may affect patient compliance, and further investigation is necessary to extend its duration of activity.

2.2. Polymers Nanoparticles and Microparticles (MPs)

Polymers have become the most widely studied drug delivery system for anti-VEGF drugs in recent years due to their versatility and tunability. They can encapsulate a wide range of hydrophilic and hydrophobic molecules, from peptides to biological macromolecules. Their pharmacokinetic characteristics can be adjusted by modifying their composition, ratios, and combining different biomaterials. Polymers offer several advantages, including biodegradability, non-toxicity, and the ability to customize their environmental, release, and retention rate features. They can be natural, synthetic, or a combination thereof. The most successful formulas are bevacizumab-loaded PLGA and chitosan-based nanoparticles.

PLGA-based nanocarriers are a versatile and promising synthetic option for drug delivery, owing to their biocompatibility, nontoxicity, degradability, and FDA-approved status. They consist of a hydrophobic core for carrying the drug and a hydrophilic outer shell (corona) that controls drug release. This amphiphilic nature makes them compatible with a wide range of drugs, enhancing their utility.

In vitro studies have shown that PLGA microspheres developed by Tanetsugu et al. can deliver ranibizumab biosimilar with more than 80% drug release achieved after three weeks [20]. Additionally, the DDS has been observed to inhibit tube formation in HUVECs. The microsphere fully degrades within 1.5 months, making it a promising candidate for prolonged anti-VEGF drug release treatment.

Sousa et al. developed PLGA-based nanoparticles loaded with bevacizumab, which showed a prolonged drug release time and preserved drug bioactivity in vitro [21][22]. They also demonstrated that bevacizumab could be stored for over 6 months while retaining its angiogenic effect using a lyophilized protocol. In another study, Zhang et al. encapsulated bevacizumab in PLGA-based nanoparticles, which were more efficient than free bevacizumab in inhibiting HUVEC proliferation and tube formation in vitro [23]. In vivo experiments on mouse models showed increased drug bioactivity in inhibiting CNV and RNV angiogenesis, with no reported toxicity or cytotoxicity. These findings suggest that PLGA-based nanoparticles loaded with bevacizumab may represent a safe and effective treatment option.

Kelly et al. investigated the use of PLGA nanoparticles to deliver aflibercept for retinal diseases treatment [24]. The study demonstrated that the DDS exhibited high encapsulation efficacy and prolonged drug release up to seven

days. On day seven, 75% of the drug was released with the DDS, compared to 100% drug release after 24 h following a standard aflibercept injection. These results show the potential of the polymer as a promising nanocarrier for delivering aflibercept.

Nanocarriers have been shown to improve anti-CNV activity. Yan et al. developed a novel nanocarrier for AMD treatment composed of PLGA-PEGylated magnetic nanoparticles, which demonstrated increased antiangiogenic efficacy [25]. The magnetic nanoparticles conferred several advantages to the DDS, such as stability, biocompatibility, and tunable surface modification. Additionally, the PEG-PLGA copolymer tested in vitro showed effective antiangiogenic activity, making this DDS a promising therapy for AMD.

Liu et al. demonstrated the potential benefits of combining multiple drugs within nanocarriers. They developed a novel poly (D, L-lactide-co-glycolide) and polyethylenimine nanoparticle that was loaded with dexamethasone and had bevacizumab added to the nanoparticle surface (eBev-DPPNs) [26]. The eBev-DPPNs were shown to effectively inhibit HUVECs angiogenesis and VEGF secretion in vitro. Furthermore, when injected intravitreally in rabbit laser models of CNV, the nanotherapeutic significantly decreased CNV leakage areas after 28 days. This demonstrates the potential of this DDS in treating CNV in vivo.

Heljak et al. developed a computational model to predict the behavior of intravitreally injected PLGA bevacizumab loaded microspheres, complementing the in vivo and in vitro studies [27]. The model successfully predicted the experimental results, indicating its potential for assessing and planning anti-VEGF treatments in clinical settings.

PLGA-based nanocarriers have shown promise for prolonged drug release. However, degradation of PLGA microspheres due to accumulation of lactic and glycolic acids can denature the drug, leading to complications. To address this issue, Liu et al. prepared a polymeric blend of poly (d, l-lactide-co-glycolide)/poly(cyclohexane-1,4-diyl acetone dimethylene ketal) (PLGA/PCADK) to deliver bevacizumab-dextran using a solid-in-oil-in-water (S/O/W) emulsification technique [28]. This novel polymer blend demonstrated increased biocompatibility compared to PLGA alone and limited the initial burst release to ensure sustained drug release. The DDS delivered drugs over a 50-day period in vitro and in vivo in a rabbit model, demonstrating the potential for this nanocarrier to become a long-term anti-VEGF treatment option.

Tsujinaka et al. developed a promising nanocarrier by using a polymer blend of PLGA-PEG to deliver sunitinib [29]. After intravitreal injection, the DDS formed a depot that impressively released drug for 6 months in a laser-induced CNV mouse model, which successfully suppressed CNV over the drug release period. The nanotherapeutic also showed potential in progressive DR therapy as it reduced VEGF-induced leukostasis and nonperfusion in a different mouse model.

Jiang et al. explored the use of polydopamine (PDA) nanoparticles as a nanocarrier for bevacizumab to treat AMD [30]. In addition to its antiangiogenic activity, the biodegradable nanocarrier reduced reactive oxygen species (ROS) and successfully delivered bevacizumab in vitro and on ex-vivo porcine eyes. The DDS has the potential to become a practical dual system for delivering antiangiogenic drugs while minimizing ROS production.

Cai et al. developed a novel drug delivery system (DDS) using modified S-PEG polymers with arginine-glycine-aspartic acid (RGD) peptide to deliver anti-VEGF agents intravenously [31]. The nanoparticles exhibited antiangiogenic activity in vitro and effectively decreased CNV lesion areas in laser-induced CNV mouse models. Moreover, the nanoparticles displayed good specificity by spending minimal time in the entire organism and by not accumulating in organs other than CNV areas, indicating their biosafety.

Natural polymers are attractive candidates for anti-VEGF nanocarriers due to their easy degradability. Although the intravitreal route remains the most common method of anti-VEGF administration, HSA nanoparticles have been explored as a potential topical route for drug delivery. HSA nanoparticles are simple to prepare and adhere well to the corneal epithelium, resulting in sustained drug bioavailability without any reported toxicity. In a study by Luis de Redin et al., bevacizumab was loaded onto HSA nanoparticles, resulting in a 13% increase in loading capacity compared to nanoparticles cross-linked with glutaraldehyde. The initial burst release of the drug was evaluated to 35% of the loaded drug within the first five minutes in vitro, with a decreased rate over the next 24 hours. In rats, the DDS was released over 4 hours before being evacuated in the gastrointestinal tract. In vivo, bevacizumab-nanoparticles showed a better antiangiogenic activity in CNV rat models than bevacizumab alone, reducing the required drug dosage by 2.4 times. The nanoparticles also significantly improved bevacizumab neovascularization inhibiting efficacy and decreased fibrosis, inflammation, and edema in rats treated with them. Although the results of this study are promising, further validation on animal models that can be easily transposed to human eye anatomy is necessary.

Llabot et al. also investigated the use of HSA nanoparticles to deliver anti-VEGF agents topically, but with the addition of Gantrez® ES-425 polymer as a stabilizing coating [32]. The nanoparticles were tested in vitro and designed to treat CNV. Gantrez® polymer was found to be a better stabilizing agent than glutaraldehyde, with improved drug stability and preserved bioactivity. In vitro release studies showed that suramin was released faster than bevacizumab, with 80% released within 8 h compared to 50% for bevacizumab. In vivo animal studies for CNV treatment with these nanoparticles are yet to be conducted.

Abdi et al. investigated the potential of chitosan nanoparticles as a carrier for bevacizumab and found that the two had minimal interactions, allowing for efficient capture and release of the drug. The study suggested that the combination of bevacizumab and chitosan nanoparticles could be a promising nanocarrier approach.

Several studies have utilized chitosan nanoparticles as a drug delivery system (DDS) for sustained drug release. Pandit et al. developed a subconjunctival injection of bevacizumab loaded PLGA nanoparticles coated with chitosan, which reduced initial drug burst release to 25%. In vitro studies showed that the DDS extended drug residency in the retina and sustained drug release for 72 h. Similarly, Ugurlu et al. administered chitosan particles loaded with bevacizumab through subtenon injection in rabbits' eyes, resulting in a sustained drug release for 3 weeks in vitro. However, in vivo results decreased within one week despite better control and progressive drug release from the DDS. Savin et al. synthesized bevacizumab loaded chitosan grafted-poly(ethylene glycol) methacrylate nanoparticles, which successfully released bevacizumab in vitro for 14-30 days. Overall, chitosan nanoparticles have shown promise as a suitable DDS for sustained drug release.

Jiang et al. developed a chitosan-based nanocarrier capable of releasing drugs over months to treat AMD. Their polycaprolactone (PLA) chitosan bi-layered hybrid shell capsule can load high drug amounts and impressively released drug over one year while preserving drug potency. Jian et al. also developed a novel DDS combining natural and synthetic polymers to carry bevacizumab in microparticles. Their chitosan-polycaprolactone core-shell microparticles increased loading capacity by 25% and decreased initial burst release to nearly 30%, while maintaining drug potency for six months. The nanotherapeutic was biocompatible with over 90% cell viability. In vivo studies remain needed to assess safety and drug efficacy.

Chaharband et al. investigated gene delivery therapy using chitosan-hyaluronic acid nano-polyplexes to deliver VEGFR-2 siRNA intravitreally in a rabbit and rat laser model of CNV [33]. The DDS was found to effectively suppress VEGFR-2 expression by 70% in vitro and significantly reduce CNV in vivo after 14 days. Although gene delivery therapy remains limited by the 1-month drug release threshold, the study suggests that the chitosan-hyaluronic acid nano-polyplexes could become an efficient intravitreal gene delivery therapy.

2.3. Lipid-Based

Formica et al. developed a hybrid lipid-based nanocapsule for co-loading bevacizumab and triamcinolone acetonide. This approach offers the potential for more effective treatment of diseases by reducing inflammation and neovascularization. The nanocarrier was shown to inhibit capillary formation in vitro, demonstrating its potential for loading multiple drugs [34].

Liposomes are a promising drug delivery system due to their hydrophilic core and hydrophobic outer shell that can be modified to improve tissue penetration. However, interactions with macrophages, pH changes, and enzymes can affect their performance, making it challenging to predict their physiological behavior accurately. In a study by Mu et al., multivesicular liposomes (MVLs) were used to encapsulate bevacizumab. The MVLs had a size ranging from 1 to 100 µm, which enabled them to avoid being captured by macrophages and to be rapidly degraded. The liposomes showed minimal toxicity, good biocompatibility, encapsulation efficacy, and low immunotoxicity. In vitro, bevacizumab was released by diffusion and erosion while maintaining its integral structure. In the laser-induced CNV rat model, the nanocarrier sustainably released the drug, and after 28 days of treatment, the DDS inhibited CNV lesions. Compared to bevacizumab solution, the DDS could potentially reduce the frequency of intravitreal injections.

Kayland Karumanchi et al. developed liposomes to encapsulate bevacizumab, achieving even longer drug release than previous studies. In vivo results showed that the DDS maintained therapeutic levels of the drug for 22 weeks, compared to less than 6 weeks for bevacizumab solution, while preserving drug potency. This suggests that liposomes have potential for prolonged and controlled drug release [35].

Liposomes have also shown potential in cancer treatment. De Cristo Soares Alves et al. developed a chitosan-coated lipid core nanocapsule for the delivery of bevacizumab to treat solid tumors, such as glioblastoma [36]. In vitro studies showed that the drug-loaded nanocarrier induced significantly more apoptosis than bevacizumab

alone. In addition, in the chicken embryo chorioallantoic membrane (CAM) assay, the nanocarrier required 5.6 times less bevacizumab than the bevacizumab solution to exhibit higher antiangiogenic effects. This suggests that the nanocarrier may reduce the toxicity and adverse effects associated with high drug doses. The results of this study suggest that this DDS has the potential to be used in the treatment of solid ocular tumors.

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