REVIEW

An Update on Novel Ocular Nanosystems with Possible Benefits in the Treatment of Corneal Neovascularization

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Abstract: Corneal neovascularization (CNV) is an ocular pathological change that results from an imbalance between angiogenic factors and antiangiogenic factors as a result of various ocular insults, including infection, inflammation, hypoxia, trauma, corneal degeneration, and corneal transplantation. Current clinical strategies for the treatment of CNV include pharmacological treatment and surgical intervention. Despite some degree of success, the current treatment strategies are restricted by limited efficacy, adverse effects, and a short duration of action. Recently, gene-based antiangiogenic therapy has become an emerging strategy that has attracted considerable interest. However, potential complications with the use of viral vectors, such as potential genotoxicity resulting from long-term expression and nonspecific targeting, cannot be ignored. The use of ocular nanosystems (ONS) based on nanotechnology has emerged as a great advantage in ocular disease treatment during the last two decades. The potential functions of ONS range from nanocarriers, which deliver drugs and genes to target sites in the eye, to therapeutic agents themselves. Various preclinical studies conducted to date have demonstrated promising results of the use of ONS in the treatment of CNV. In this review, we provide an overview of CNV and its current therapeutic strategies and summarize the properties and applications of various ONS related to the treatment of CNV reported to date. Our goal is to provide a comprehensive review of these considerable advances in ONS in the field of CNV therapy over the past two decades to fill the gaps in previous related reports. Finally, we discuss existing challenges and future perspectives of the use of ONS in CNV therapy, with the goal of providing a theoretical contribution to facilitate future practical growth in the area.
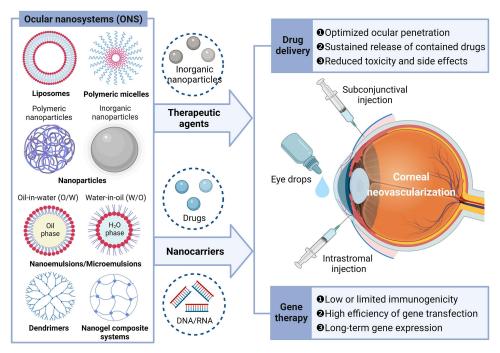
Keywords: ocular nanosystems, nanocarriers, corneal neovascularization, drug delivery, gene therapy

Introduction

Corneal neovascularization (CNV), also called corneal angiogenesis, involves the formation of new vascular structures in transparent cornea areas that were previously avascular. Such pathological changes can lead to tissue scarring, edema, lipid deposition, and persistent inflammation that may significantly affect visual prognosis and quality of life if not addressed promptly.[1] In addition, since it is a crucial risk factor for corneal graft rejection, its treatment before any keratoplasty procedure is essential.[2] Corneal angiogenesis results from an imbalance between angiogenic and antiangiogenic factors that preserve corneal transparency (Created with BioRender.com) as a result of various ocular insults, including infection, inflammation, hypoxia, trauma, corneal degeneration, and corneal transplantation.[1,3] Current clinical treatment strategies for CNV include pharmacological treatment, such as the use of anti-inflammatory and immunosuppressive agents and vascular endothelial growth factor (VEGF) inhibitors, or surgical intervention, including laser ablation, photodynamic therapy (PDT), and fine-needle diathermy (FND).[4] Despite some degree of success, the current treatment strategies are restricted by limited efficacy, adverse effects, and a short duration of action.[5] Recently, gene-based antiangiogenic therapy has become an emerging strategy that has attracted considerable interest.[6] However,

Graphical Abstract

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potential complications with the use of viral vectors, such as potential genotoxicity resulting from long-term expression and nonspecific targeting, cannot be ignored.[7]

Nanotechnology is a scientific nanoscale engineering technology that involves elements at the nanometer scale. By applying nanostructures in various fields of science, nanotechnology has been shown to bridge the barriers of biological and chemical-physical sciences.[8] To date, nanotechnology has been used in almost all areas of medical science, including imaging, diagnostics, biosensors and drug delivery.[9] The use of nanotechnology-based novel ocular nanosystems (ONS) in various ocular disease treatments has been investigated for the last two decades.[10,11] The potential functions of ONS range from nanocarriers—which deliver drugs and genes to the targeted site of the eye—to therapeutic agents themselves. The novel ONS may offer new perspectives in the treatment of ocular diseases by realizing targeted delivery, allowing controlled release, ensuring low eye irritation, improving drug bioavailability or enhancing ocular tissue compatibility.[12]

Recent advancements in the field of ONS could circumvent the above limitations existing in the current therapeutic strategies for CNV, and various preclinical studies conducted to date have demonstrated promising results of ONS in the treatment of CNV. In this review, we provide an overview of CNV and its current therapeutic strategies and summarize the properties and applications of various ONS related to the treatment of CNV reported to date. Our goal is to provide a comprehensive review of these considerable advances in ONS in the field of CNV therapy over the past two decades to fill the gaps in previous related reports. Finally, we provide considerations around current challenges and future perspectives of the use of ONS in CNV therapy, with the goal of providing a reference for the clinical translation of ONS.

Formation of CNV

A healthy cornea is an avascular transparent tissue nourished by diffusion from the aqueous humor and tear film. The term "angiogenic privilege" is used to describe the maintenance of corneal avascularity,[13,14] which means that, in the resting state, healthy corneas can exist in an active process involving the homeostatic balance between low levels of angiogenic and high levels of antiangiogenic factors.[15] Once such delicate equilibrium is disturbed by various ocular insults, including infection,

inflammation, hypoxia, trauma, corneal degeneration, and corneal transplantation, abnormal new vessels can invade the corneal stroma from preexisting pericorneal structures and subsequently lead to CNV.[16] Furthermore, this CNV can disrupt the relative immune privilege of the cornea, causing a positive feedback cycle of inflammation and more CNV.[3] Corneal pathologies that can lead to neovascularization include lipid keratopathy, corneal ulcers and scars, herpes eye disease, infectious keratitis, chemical burns, graft rejections and hypoxic insults from contact lens wear.[4]

As mentioned above, multiple pathologic insults may result in CNV. These pathologies can be broadly thought of as initiating at least one of two common pathways: (1) loss of the limbal stem cell barrier and (2) inflammation.[3] Inflammation is a central pathological process in the formation of CNV. Many articles have discussed inflammatory mechanisms contributing to CNV.[3,17] In the following, we provide a brief introduction of the inflammatory mechanisms contributing to CNV (Figure 1).

When the cornea is damaged, multiple cell types, such as corneal epithelial and endothelial cells, stromal keratocytes, immune cells, vascular endothelial cells (VECs), and pericytes, release several proangiogenic cytokines, including VEGF, basic fibroblast growth factor (bFGF), chemokines, and adhesion molecules. Moreover, this upregulation of
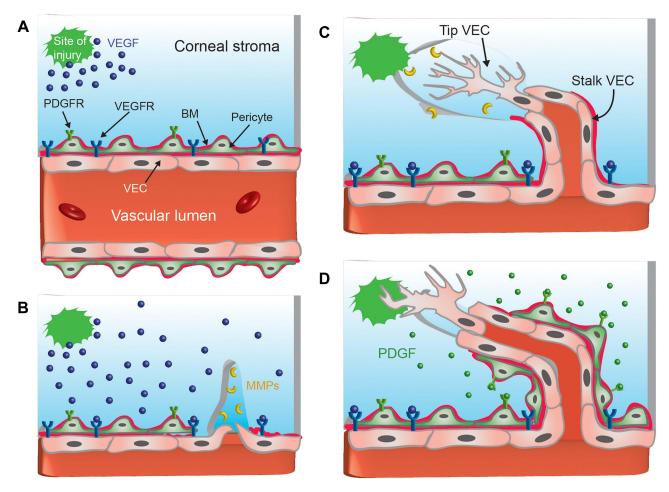

Figure 1 Simplified schematic pathway of inflammatory CNV.
Notes: (**A**) A limbal vessel is shown in cross section. Corneal injury leads to production of proangiogenic substances including VEGF, bFGF, and cytokines by a variety of cell types (only VEGF is shown here for simplicity). (**B**) Activation of VEGFR causes pericyte loss and release of MMPs from VECs, leading to destruction of the VEC BM and surrounding ECM of the cornea, and allowing VECs to migrate into the cornea via chemotaxis. (**C**) Migration and proliferation of VECs leads to sprouting of a neovascular stalk that invades the corneal stroma. The cells at the tip of this stalk form dendritic projections as they move chemotactically toward the site of inflammation, and continue releasing MMPs. VECs begin secreting BM. The new vessel is tenuous due to lack of pericyte support. (**D**) As neovascularization progresses, VECs release PDGF, which binds to receptors on pericytes, leading to their proliferation and migration. Pericytes then associate with and stabilize the new vessel. Reproduced from Nicholas MP, Mysore N. Corneal neovascularization. *Exp Eye Res*. 2021;202:108,363. © 2021 Published by Elsevier Ltd. With permission from Elsevier.[3]
Abbreviations: CNV, Corneal neovascularization; BM, basement membrane; ECM, extracellular matrix. MMP, matrix metalloproteinase; PDGF, platelet derived growth factor; PDGFR, PDGF receptor; VEC, vascular endothelial cell; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

proangiogenic factors is accompanied by the downregulation of antiangiogenic factors such as pigment epithelium-derived factors. The activation of vascular endothelial growth factor receptor (VEGFR) leads to the release of matrix metalloproteinases (MMPs) from VECs, which degrade the vascular basement membrane and surrounding corneal extracellular matrix. In this context, VECs are able to migrate and proliferate into the cornea, causing neovascular stalk sprouting and resulting in invasion of the corneal stroma. The cells at the tip of this stalk form dendritic projections as they move chemotactically toward the site of inflammation and continue releasing MMPs. As neovascularization progresses, VECs release platelet-derived growth factor, causing the proliferation and migration of pericytes. Finally, the new vascular lumen matures and is stabilized as supporting pericytes are recruited, indicating that abnormal new vessels do not require proangiogenic factors for survival.

Strategies for the Treatment of CNV and Corresponding Limitations
Pharmacological Treatment

Pharmacological treatments need to deliver drugs to the eyes. Topical eye drops or subconjunctival injections have been considered conventional delivery routes for anterior segment diseases. However, human eyes have various defensive barriers (such as tear film, cornea, and blood barriers) and clearance mechanisms (such as blinking reflex, tear renewal, and tear drainage).[18] Hindered by these ocular barriers and factors, the efficacy of the total amount of administered drugs is less than 5%, thus rendering the poor bioavailability of ocular drugs.[19] Low drug penetration, transient residence time at the targeted site, and potential side effects resulting from frequent administration are also common drawbacks with respect to conventional drug delivery.[20]

To date, there are a wide range of drugs for CNV treatment. According to the properties and targets of drugs, they can be divided into different types, which have been reviewed elsewhere.[3,6,21,22] We list several important and emerging drugs below.

Anti-Inflammatory Drugs

Steroids, including dexamethasone (DEX), are potent inhibitors of inflammation and have been used in the treatment of CNV for their anti-inflammatory[23] and antiangiogenic[24] properties. Steroids are most effective in inhibiting CNV when started before or immediately after tissue injury. However, the complete suppression of CNV with topical steroids is not possible, as steroids do not cause established CNV to regress.[25] Moreover, steroids have various potential side effects, such as steroid-induced glaucoma, acceleration of cataract formation, superinfection, and herpes simplex recurrence.[26] Therefore, it is crucial to closely monitor patients on long-term steroids for early diagnosis and management of potential side effects.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are also anti-inflammatory agents with known antiangiogenic properties, but they are not considered sufficiently effective.[27,28] Notably, similar to steroids, long-term use of topical NSAIDs may cause potential corneal side effects that necessitate close monitoring.[29]

Immunosuppressants

Cyclosporine A (CsA) is an immunosuppressive agent used to control the rejection of organ transplants and to treat multiple autoimmune and inflammatory conditions.[30] A study indicated that topical 0.05% CsA shows an inhibitory effect on immune-mediated CNV in rabbits, with the inhibitory effect of topical 0.05% CsA treatment being significantly higher than that of topical isotonic saline or 0.5% bevacizumab (BEV) (the percent area of CNV was 24.4 ± 14.4, 37.1 ± 20.4 and 44.1 ± 25, respectively).[31] Notably, systemic administration of CsA in high-risk corneal transplantation led to side effects, including eczema, back pain, chest pain, and nausea.[32] The side effects of CsA for CNV treatment need further clinical research.

Tacrolimus is another potent immunosuppressant that effectively blocks the production of cytokines by T cells and immunoglobulins by B cells.[22] Systemic, topical and subconjunctival tacrolimus application has been shown to be useful in reducing experimental CNV.[33,34]

Anti-VEGF Drugs

Suppressing VEGF activity with anti-VEGF antibodies such as BEV is a possible strategy for CNV treatment.[35] BEV is a full-length humanized murine monoclonal IgG1 antibody that can recognize all isoforms of VEGF-A.[3] Topical BEV has

been successfully utilized for the reduction in CNV in cases unresponsive to conventional anti-inflammatory medications in animal studies and clinical trials.[22,36] Apart from topical administration, subconjunctival BEV application also achieves a significant reduction in the area of CNV. A significant reduction in CNV area was seen in clinical human studies, with a pooled reduction of 36% [95% confidence interval (CI), 18–54%] overall, of 48% (95% CI, 32–65%) for topical treatment, and 32% (95% CI, 10–54%) for subconjunctival BEV injections.[37] Notably, topical administration of BEV may lead to an increased risk of corneal epithelial defects in a time- and dose-dependent manner, while subconjunctival administration has been widely used in clinical studies with no obvious side effects.[21] However, CNV may recur following successful management with subconjunctival BEV necessitating repeated injections, especially in cases with lipid deposition.[38] In addition, similar to steroids, anti-VEGF antibodies are less effective in treating mature CNV.[3]

VEGFR tyrosine kinase inhibitors, such as apatinib, cabozantinib and axitinib, can inhibit the combination of receptors and ligands by interacting with the starting point of VEGF signal transduction pathways. It can suppress the formation of CNV that is mediated by VEGF.[39–41]

Plant Extracts

Several plant extracts, including epigallocatechin gallate (EGCG),[42] celastrol,[43] curcumin,[44] kaempferol[45] and naringenin,[46] have been found to inhibit experimental CNV. The plant extracts mentioned above (except EGCG, which is a water-soluble active ingredient) are all hydrophobic molecules. Therefore, despite their powerful beneficial bioactivity, their clinical use has been limited mainly due to their poor water solubility. In this context, seeking a next-generation delivery carrier/strategy becomes an urgent issue to improve their water solubility while still retaining their activities in the treatment of CNV.

Surgical Treatment

Physical ablation with lasers, PDT and FND may be considered when the pharmacological treatments described above show no effect, especially in the setting of relatively mature vasculature. The applications of these techniques in the treatment of CNV have been reviewed by other authors.[4,22,27] Although the above surgical treatments have shown potential in a clinical setting, they all may evoke an inflammatory response, which can further exacerbate CNV.[47] Moreover, in the laser ablation procedure, high laser energy can predispose patients to various complications, including iris atrophy, corneal thinning, pupillary ectasia, peripheral corneal hemorrhage[48,49] and necrotizing scleritis.[50] Considering the fact that there may be collagen shrinkage and damage to the adjacent stroma of the diathermy site as well as the fact that the long-term effects of FND on the cornea are not yet clear,[6] the use of FND has also not gained widespread acceptance.

Gene Therapy

The unique anatomical position and immune privilege of the cornea along with the relative ease of access enable it to be a promising candidate for gene-based therapy—either through transgenic expression of an antiangiogenic factor or inactivation of a proangiogenic factor via gene silencing. Unlike pharmacological or surgical treatment for CNV that only provides limited efficacy, a gene-based strategy offers targeted treatments providing long-term therapeutic correction.[51] Studies have suggested that antiangiogenic genes such as endostatin and angiostatin can be transfected into corneal epithelial cells, successfully reducing CNV in animal models.[52–54] In addition, small interfering RNA (siRNA) targeting VEGFs has been demonstrated to inhibit CNV in animal models.[55] However, gene-based therapies for corneal neovascularization are still largely at the preclinical stage. The success of gene therapy seen in some animal studies is accomplished by early and frequent administration, which is far from ideal for treating ongoing CNV.[6]

Liu et al[6] provided a comprehensive review of therapeutic target genes and potential gene carriers available (eg, adenoviral vectors, lentiviral vectors, adeno-associated viral vectors and nanocarriers) to treat CNV. Although viral-based vectors are well established and effective, there are safety concerns, including the potential genotoxicity of long-term expression and nonspecific targeting.[7] Furthermore, lentiviral vectors randomly integrate their genome into host cells, which can lead to insertional mutagenesis.[56] Therefore, novel nanocarriers and nonviral delivery methods that are less likely to induce an immune response and can realize targeted gene delivery might offer considerable benefits in ocular gene delivery in the future.

Different Forms of ONS for CNV Therapy

To address these limitations of current treatment strategies in CNV, novel ONS based on nanotechnology have been investigated for the last two decades. The purpose of these ONS is not only to optimize ocular penetration and bioavailability, but also to prolong drug retention time, thereby achieving sustained delivery and controlled therapeutic release with minimal toxicity and side effects.[19] For gene therapy, ONS also provide more biocompatible nanovectors with high efficiency of gene transfection and long-term gene expression, which exert considerable benefits in the treatment of CNV. To date, the use of various ONS, such as liposomes, nanoparticles (NPs), polymeric micelles, nanoemulsions/microemulsions, dendrimers, nanogel composite systems (Figure 2) and nanowafers, has become a popular research topic in the field of CNV therapy.

Other ONS, such as nanosuspensions,[57] cubosomes,[58] and spanlastics[59] are also promising ocular delivery platforms. However, to date, there have been no documented studies reporting their applications in the treatment of CNV. Therefore, these ONS are not discussed in this review, as the main focus of this review is CNV rather than a wider range of eye diseases. The properties of various ONS closely related to CNV are summarized below.

Liposomes

Liposomes are spherical vesicles formed by one or more phospholipid bilayers.[60] They are formulated primarily from phospholipids and cholesterol, which enable liposomes with not only high ocular penetration but also satisfactory biocompatibility, biodegradability, almost no toxicity and low antigenicity.[61] Liposomes can be used to simultaneously load both hydrophilic and hydrophobic molecules. Hydrophilic molecules can be encapsulated into the core region, while hydrophobic molecules can be encapsulated in lipid bilayers.[62] Currently, liposomes are widely used for the delivery of different drugs,[60] therapeutic agents[63,64] and genes[65] to the eye.

The applicability of liposomes as ONS in specific ocular diseases is determined by their lipid composition, preparation approaches and surface charge.[66] For instance, the absorption of encapsulated drugs across corneal membranes can be enhanced by positively charged liposomes.[67] In addition, via electrostatic interactions, chitosan-coated deformable liposomes with positive charges have the capacity to improve loading efficacy and to enhance binding affinity with negatively charged corneal surfaces.[68]
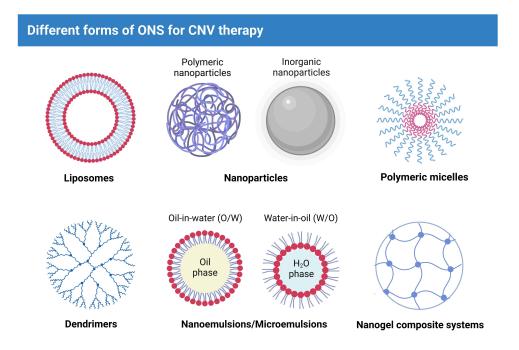

Figure 2 Different forms of ONS for CNV therapy. Created with BioRender.com.

However, despite liposomes having the considerable advantages mentioned above, the low stability, relatively low entrapment, and rapid release of hydrophilic drugs substantially limit the advancement of liposomes in clinical applications. In response, many studies have been conducted in efforts to enhance liposome bioavailability, corneal penetration, stability, and targeted action and have achieved positive results.[69,70]

NPs

NPs are nanoscale colloidal carriers that are mainly made of natural or synthetic polymer composition, inorganic materials, lipids and proteins and peptides.[71,72] NPs can be coated with a hydrophilic polymer or further functionalized with antibodies modified onto the coating.[71] In addition, they can be formulated with different sizes, charges, solubilities and other physicochemical characteristics, conferring great versatility in terms of the kind of therapeutic molecules to be loaded. To date, NPs have been investigated in the field of ocular delivery and show great promise as novel ONS in the treatment of ocular diseases.[73] NPs can be divided into polymeric NPs and inorganic NPs on the basis of their composition.

Polymeric NPs

For polymeric NPs, commonly used materials include poly(lactide-co-glycoside) (PLGA), poly(lactide) (PLA), poly(ε-caprolactone) (PCL), dextran, albumin, gelatin, alginate, collagen, hyaluronic acid (HA), and chitosan.[71] The possibilities for their design are nearly limitless.

Among the various polymers developed to formulate polymeric NPs, PLGA has been extensively investigated as an ONS in the field of ocular delivery due to its biodegradability, biocompatibility and nonantigenic nature. PLGA also provides protection of the drug from degradation and the possibility of sustained release. Sánchez-López et al[74] successfully developed the PLGA NPs formulation with a high drug entrapment efficiency of greater than 85% and presented a sustained drug release profile compared to that of conventional eye drops. The positively charged surface of PLGA NPs could be used to uplift the penetration of various drugs, such as DEX[75] and pranoprofen,[76] into the cornea. Therefore, PLGA NPs have been widely applied in drug delivery to reduce the frequency of drug administration for ocular disease treatment.[77]

Gelatin is a natural biopolymer prepared and purified from collagen. Gelatin nanoparticles (GNPs) have excellent biocompatibility and biodegradability; hence, they have been previously used as carriers with reported successful drug/gene delivery in ophthalmic applications.[77] Since collagen is the major component of corneal stroma, the use of GNPs as the drug carrier in eye drop formulations can improve the bioavailability of drugs or genes by interacting with corneal and conjunctival glycoproteins.[78]

The increasing use of polymeric NP formulations to increase the cargo molecule residence time inside the cornea with a high permeation rate provides a strong rationale for their translation and clinical application in ocular delivery. However, the limitations posed by their stability, particle size uniformity, release kinetics and bulk production issues should also be considered.[62]

Inorganic NPs

Common materials used for the production of inorganic NPs include gold,[79] silver,[80] silica[81] and cerium oxide.[82]

Gold nanoparticles (AuNPs) are promising platforms for biomedical applications, especially for drug delivery[83] and gene delivery,[84] because of their amenability to synthesis, stabilization, and functionalization; low toxicity; and ease of detection.[85] Apart from being employed as effective drug or gene carriers, AuNPs have also been shown to act as anti-angiogenesis agents to inhibit angiogenesis and vascular permeability through the downregulation of VEGFR-2 expression.[86] Similar to AuNPs, silver nanoparticles (AgNPs) conjugated with a heparin derivative have demonstrated efficacy as antiangiogenic agents.[87]

The porous property of mesoporous silica allows a high surface area and high pore volume to absorb and encapsulate molecules.[88] Moreover, due to their small size (5–50 nm), silica nanoparticles (SiNPs) can effectively increase penetration into the cornea and provide further access to the vitreous area.

In recent years, cerium oxide nanoparticles (CeNPs) have gained increasing attention in the context of biomedical applications. CeNPs can clear superoxide radical anions, conferring good reduction capability.[89] Hence, CeNPs can act as excellent antioxidants to reduce oxidative stress for the treatment of ocular diseases. To date, CeNPs have been used in biomedical materials for vascular inhibition and treatment of photoreceptor degeneration.[90–92]

However, these inorganic NPs are not biodegradable, leading to a potential issue in biological toxicity. It is critical to develop and/or identify appropriate in vitro and in vivo models to assess the toxicity of these NPs.

Polymeric Micelles

Polymeric micelles are nanostructures formed by block copolymers with amphiphilic properties. The lipophilic portion comprises a micelle core that encapsulates the hydrophobic molecules, while the hydrophilic part comprises the outer surface of the micelle to increase cargo molecule solubility and stabilize and prolong the half-life of the therapeutic agents.[93] In addition, polymeric micelles are biodegradable and biocompatible, thus preventing adverse effects.[47] Currently, polymeric micelles have attracted considerable interest as ONS and offer significant benefits in ocular delivery.

Various studies have demonstrated that the amphiphilic nature of polymeric micelles enables them to penetrate lipophilic corneal epithelial and endothelial cells, and they can also penetrate a hydrophilic matrix, thus promoting drug penetration and improving bioavailability.[94–96] In addition, the small size of polymeric micelles might enhance paracellular transport through conjunctiva and sclera, resulting in higher drug levels in intraocular tissues and a sustained drug delivery effect.[97] For gene therapy, an in vitro study showed prolonged gene expression after subconjunctival injection of polymeric micelles containing a reporter gene. In addition, gene transfer into the subconjunctival space by polymeric micelles showed significant inhibition of CNV in mice.[98] Importantly, polymeric micelles have been shown to have no cytotoxicity in human corneal epithelial cells.[99] Based on these abovementioned advantages, polymeric micelles are believed to be safe ONS for treating corneal diseases.

Nanoemulsions/Microemulsions

Nanoemulsions contain an oil phase, water phase, emulsifier, and coemulsion. The presence of surface active ingredients in nanoemulsions enables enhanced mixing of nanosized droplets with the precorneal constituents and, as a consequence, a greater dispersion of the drug over the cornea.[100] Microemulsions are isotropic, transparent, and thermodynamically stable nanosized mixtures of oil, water, surfactant and cosurfactant. The small size of the microemulsions and the presence of surfactant among the components confer properties such as good tissue permeability[101] and improvement in solubility and stability of the applied drug.[102] Thus, reliable patient compliance is attained, and the number of administrations needed decreases daily.[103] Nanoemulsions and microemulsions are currently widely being investigated as ONS for ocular delivery, primarily to the front of the eye.

The properties of nanoemulsions and microemulsions depend on the nature and composition of their components. Kalam's work revealed that the optimized microemulsion possessed good stability, showed greater adherence to the corneal surface and good permeation of gatifloxacin in the anterior chamber of the eye, resulting in a twofold higher gatifloxacin concentration than that of the conventional dose.[101]

However, microemulsions are unsuitable when the therapeutic molecules are water-soluble or insoluble (does not dissolve in water or oil) or thermolabile or if the therapeutic molecules appear transparent externally. In addition, unlike liposomes, microemulsions are unsuitable for long-term sustained drug release.[61]

Dendrimers

Dendrimers are repeated/individual molecules with a regularly branched structure that contain many sidechain moieties arranged in a highly regular branching pattern, typically symmetrically around a central core. Owing to their regularly arranged framework, the unique branched topologies of dendrimers afford properties such as high solubility, availability of tremendous internal cavities loaded with various therapeutic molecules, and controllable molecular weight.[70] To date, dendrimers have been widely used as ONS for the delivery of different drugs and therapeutic agents to the eye.[104,105]

Poly(amidoamine) (PAMAM) dendrimers are most commonly used among dendrimers for ocular delivery due to improved biological response, tolerability and relatively low clearance from the ocular surface.[106] In addition, PAMAM

dendrimers can not only solubilize drugs that have poor water solubility but also increase the ocular residence time upon topical administration.[107] Moreover, in vitro studies have revealed that the interactions between negatively charged ocular mucins and PAMAM dendrimers can lead to subsequent intensified corneal penetration.[108]

Nonetheless, although dendrimers have several advantages, as mentioned above, they may cause chemical modifications to drug molecules, leading to cytotoxicity issues. In addition, multistep syntheses and high preparation costs have prohibited further advancement of dendrimers from the laboratory to the clinic.[104]

Nanowafers

Nanowafers are very small, transparent circular discs fabricated by different polymers (Figure 3). The composition of nanowafers enables them to be readily applied to the ocular surface with a fingertip and to withstand constant blinking without being displaced. In addition, nanowafers contain arrays of drug-loaded nanoreservoirs from which the drug is released in a tightly controlled manner for a few hours to days to enhance drug residence time and subsequent absorption into ocular tissues. Moreover, at the end of the stipulated period of drug release, nanowafers dissolve and are carried away.[109] Good tolerability and stability, together with the enhanced therapeutic effect of the drug with minimal side effects, make nanowafers ideal ONS for ophthalmic drug delivery. To date, nanowafers have offered considerable benefits in the stable delivery of axitinib for the effective treatment of CNV.[109]

Nanogel Composite Systems

To further improve the ocular therapeutic duration and bioavailability of therapeutic agents released from the above-mentioned ONS, different nanocarriers such as NPs[110,111] and dendrimers[112] have been embedded in hydrogels to form nanogel composite systems in recent years. The drug release duration of the composition system is longer than that of the nanocarriers alone and the hydrogel matrix alone. In addition, the composite system can also improve the biocompatibility of the nanocarriers by hiding them within the hydrogel and minimizing drug metabolism from the enzymes present in tears or on the corneal surface.[113]
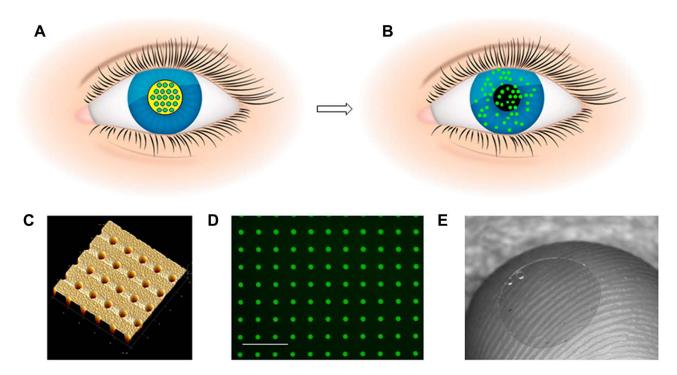

Figure 3 Ocular drug delivery nanowafer.
Notes: (**A**) Schematic of the nanowafer instilled on the cornea. (**B**) Diffusion of drug molecules into the corneal tissue. (**C**) AFM image of a nanowafer demonstrating an array of 500 nm diameter nanoreservoirs. (**D**) Fluorescence micrograph of a nanowafer filled with doxycycline (scale bar 5 μm). (**E**) Nanowafer on a fingertip. Reproduced with permission from Yuan X, Marcano DC, Shin CS, Hua X, Isenhart LC, Pflugfelder SC, Acharya G. Ocular drug delivery nanowafer with enhanced therapeutic efficacy. *ACS Nano*. 2015;24;9(2):1749–1758. © 2015 American Chemical Society.[109]

Since the formulations of nanogel composite systems are versatile and complex, the quality control of such systems is comprehensive. It is therefore necessary to consider the quality of the entire system, including the stability of the gel and the gelling performance of the in situ gel at the macro level. Importantly, due to the composition of many auxiliary materials in nanogel composite systems, safety issues should not be ignored.[93]

Applications of ONS in CNV Therapy
ONS as Nanocarriers
Drug Delivery Based on ONS

Multiple ONS have been utilized as nanocarriers for the delivery of different drugs, including DEX, CsA, tacrolimus, BEV, apatinib, cabozantinib, axitinib, plant extracts and some signaling pathway inhibitors, to the eye in the field of CNV therapy (Table 1).

Table 1 Drug Delivery Based on ONS for CNV

ONS Type	Formulation	Subjects	Models	Delivery	Dose	Results	Ref.
Liposomes	Liposomes encapsulating tacrolimus (FK506)	Mouse	Alkaline	Eye drops	0.2 mg/mL, 10 μL, QID for 14 days after injury	Reduced by 63.4% and 60.0% in vessel length and CNV area respectively. Its therapeutic effect was better than those of the commercial FK506 eye drops (1 mg/mL) and the free drug (0.2 mg/mL)	[64]
Liposomes	Liposomes encapsulating XAV939	Mouse	Alkaline	Eye drops	10 μL, BID for 14 days after injury	Reduced by 50.0% and 70.6% in vessel length and CNV area respectively	[63]
NPs	MePEG-PCL NPs encapsulating curcumin	Mouse	Alkaline	Eye drops	20 μL, QD for 14 days after injury	Reduced CNV area by 50.0%	[44]
NPs	HSA-PEG NPs loading apatinib	Mouse	Alkaline	Subconjunctival injection	124 μg/eye at day 3 and day 5 after insult	Reduced CNV area by 53.6%	[39]
NPs	PLGA NPs encapsulating BEV	Mouse	Alkaline	Subconjunctival injection	1 μg/mL after injury	Reduced vascular length by 91.1%	[117]
NPs	Albumin NPs encapsulating BEV	Mouse	Alkaline	Eye drops	4 mg/mL, 10 μL, QD for 7 days after injury	Reduced CNV area by 61.0%	[118]
NPs	PLGA NPs encapsulating DSP	Mouse	Nylon suture	Subconjunctival injection	30 μL after insult	Improved drug retention and prolonged drug levels in anterior chamber for more than 7 days and prevented CNV in rats for 2 weeks	[114]
NPs	GNPs encapsulating kaempferol	Mouse	Alkaline	Eye drops	5 μL, QD for 7 days after injury	Reduced CNV area by 80.6%	[45]
NPs	GEH-RGD NPs loading EGCG	Mouse	Alkaline	Eye drops	30 μg/mL, QD for 7 days after injury	Reduced CNV area by 52.5%	[42]

(*Continued*)

Table 1 (Continued).

ONS Type	Formulation	Subjects	Models	Delivery	Dose	Results	Ref.
NPs	MSNs encapsulating BEV	Mouse	Alkaline	Subconjunctival injection	0.01 mg BEV after injury	Enhanced the inhibitory effects of BEV on CNV and reduced vascular length by 91.3%	[119]
NPs	GNPs encapsulating 5Z-7-oxozeaenol	Mouse	Alkaline	Eye drops	5 μL, QD for 7 days after injury	Reduced CNV area by 86.4%	[124]
Polymeric micelles	MPEG-hexPLA micelles encapsulating CsA	Mouse	Transplant	Eye drops	25 μL, five times a day for 14 days after transplant	Reduced CNV area by 50.0% and improved success rate of cornea graft transplantation	[116]
Polymeric micelles	PEG-b-PCL micelles encapsulating celastrol	Mouse	Nylon suture	Subconjunctival injection	5.44 mg/mL, 0.1 mL on day 0 and 3 after injury	Reduced by 57.8% and 70.3% in vessel length and CNV area respectively	[43]
Polymeric micelles	MPEG-PCL micelles loading axitinib	Mouse	Alkaline	Eye drops	20 μL after injury	Positive effect on maintaining corneal thickness and antiangiogenic efficiently. Reduced by 38.5% and 83.3% in vessel length and CNV area respectively	[41]
Polymeric micelles	Cationic polypeptide micelles encapsulating cabozantinib	Mouse	Alkaline	Eye drops	0.5 mg/mL, 10 μL, TID for 14 days after injury	Reduced by 35.3% and 20.4% in vessel length and CNV area respectively	[40]
Polymeric micelles	p(AAPBA-r-GEA) glycopolymeric micelles loading DEX	Mouse	Alkaline	Eye drops	25 μL, QID for 7 days after injury	Reduced CNV area by 47.5% and achieved a more significant efficacy in neovascularization suppression and inflammation elimination compared to the commercialized DEX eye drops with no adverse effects on the cornea	[115]
Micro-emulsions	Microemulsions encapsulating naringenin	Mouse	Alkaline	Eye drops	5 μL, QID for 7 days after injury	Reduced CNV area by 54.4%	[46]
Nanowafer	Nanowafer loading axitinib	Mouse	Alkaline	Eye drops	5 μg, QD for 10 days after injury	Axitinib nanowafers administered once a day were therapeutically twice as effective as axitinib delivered twice a day by topical eye drop therapy	[109]
Nanogel composite system	Injectable gel incorporating dendrimer DEX conjugates	Mouse	Alkaline	Subconjunctival injection	1.6 mg/eye after injury	Reduced CNV area by 58.6% and significant suppressed pro-inflammatory cytokines production compared to free DEX.	[112]
Nanogel composite system	BEV@MSN and CsA@Thermogel	Rabbit	Alkaline	Subconjunctival injection	5 mg/mL BEV, 2 mg/mL CsA, 0.1 mL after injury	Reduced by 22.2% and 64.4% in vessel length and CNV area respectively	[111]

Abbreviations: ONS, Ocular nanosystems; CNV, Corneal neovascularization; NPs, Nanoparticles; MSNs, Mesoporous silica nanoparticles; PLGA, Poly (lactide-co-glycoside); PEG, Poly (ethylene glycol); PLA, Poly(lactide); PCL, Poly(ε-caprolactone); HSA, Human serum albumin; HSA-PEG, Human serum albumin-conjugated poly (ethylene glycol); DEX, Dexamethasone; DSP, Dexamethasone sodium phosphate; CsA, Cyclosporine A; BEV, Bevacizumab; EGCG, Epigallocatechin-3 gallate; GNPs, Gelatin nanoparticles; HA, Hyaluronic acid; RGD, Arginine-glycine-aspartic acid; GE, Gelatin/EGCG self-assembling nanoparticles; GEH-RGD, GE nanoparticles with HA-RGD surface decoration; MPEG-hexPLA, Methoxy poly(ethylene glycol)-hexylsubstituted poly(lactides); MePEG-PCL, Poly(ethylene glycol) methyl ether-block-poly(ε-caprolactone); PEG-b-PCL, Poly(ethylene glycol)-block-poly(ε-caprolactone); MPEG-PCL, Methoxy poly(ethylene glycol)-poly(ε-caprolactone); p(AAPBA-r-GEA), Amphiphilic PBA-based glycopolymer poly(3-acrylamidophenylboronic acid)-r-poly(2-O-acryloyloxyethyl-β-d-galactopyranoside); BEV@MSN, MSNs loaded with BEV; CsA@Thermogel, CsA-containing thermo-sensitive hydrogel matrix; QD, Once a day; BID, Twice a day; TID, Three times a day; QID, Four times a day.

The antiangiogenic effect of various steroids, including DEX, has been shown in multiple animal models and in clinical practice.[28] However, inhibiting and/or treating CNV requires frequent administration of steroids, which results in patient incompliance, particularly in elderly individuals. Therefore, the development of a sustained steroid delivery system to the anterior segment with high bioavailability to reduce dosing frequency is needed. PLGA NPs,[114] polymeric micelles[115] and the PAMAM dendrimer-gel composite system[112] were able to deliver DEX or dexamethasone sodium phosphate (DSP) to the mouse cornea via subconjunctival injection or topical administration. These nanoformulations achieved sustained drug release and showed more robust efficacy on CNV suppression and inflammation elimination than free DEX or DSP, with a negligible effect on normal tissues. Thus, the number of administrations needed decreases daily.[112,114]

CsA and tacrolimus are emerging immunosuppressive agents used to inhibit CNV.[31,33,34] Di et al[116] developed novel micelle formulations based on methoxy poly(ethylene glycol)-hexylsubstituted poly(lactides) (MPEG-hexPLA) copolymers and used the nanosized polymeric micelles as drug carriers of CsA in a rat model of cornea transplantation. The results showed that when applied topically, MPEG-hexPLA micelles with 0.5% CsA can significantly decrease graft rejection and reduce CNV. Lin et al[64] prepared cationic liposomal tacrolimus with a surface potential of approximately +30 mV; the authors claimed that tacrolimus liposomes (0.2 mg/mL) inhibited CNV and reduced corneal inflammation. Moreover, its therapeutic effect was better than those of the commercial tacrolimus eye drops (1 mg/mL) and the free drug (0.2 mg/mL).

The use of anti-VEGF antibodies such as BEV is a possible strategy for CNV treatment.[21,27] PLGA NPs,[117] albumin NPs[118] and mesoporous silica nanoparticles (MSNs)[119] were utilized as nanocarriers to deliver BEV to enhance antiangiogenic effects in CNV therapy. These novel strategies of encapsulating BEV could increase the bioavailability and decrease the toxicity of BEV in animal models of CNV. Recently, Lyu et al[111] successfully embedded MSNs loaded with BEV in a CsA-containing thermosensitive hydrogel matrix. This nanogel composite system can regulate the in vitro release of both BEV and CsA in a sustainable way for up to four weeks, thus enhancing CNV inhibition through synergistic anti-VEGF and anti-inflammation effects.

Apatinib, cabozantinib and axitinib are VEGFR tyrosine kinase inhibitors that have the ability to suppress CNV formation mediated by VEGF.[39–41] However, as hydrophobic drugs, their application to the ocular surface needs to be improved. To surmount the ocular surface barriers and release the drug for extended periods of time, Lee et al[39] encapsulated water-insoluble apatinib in NPs composed of human serum albumin (HSA)-conjugated poly(ethylene glycol) (PEG). The authors demonstrated that a subconjunctival injection of apatinib-loaded HSA-PEG NPs efficiently led to lower levels of alkali burn injury-induced CNV in rats than that observed with an injection of free apatinib solution. Han et al[40] developed cationic polypeptide micelles with mucoadhesive ability that carry lipophilic cabozantinib to enhance the bioavailability of lipophilic cabozantinib and remarkably inhibit CNV. Shi et al[41] used methoxy poly (ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL) to encapsulate axitinib to prepare axitinib-loaded micelles. The results showed that axitinib-loaded micelles exerted superior antiangiogenic effects with remarkable reductions in the CNV area, which were as effective as clinical DEX but without apparent side effects, offering a new prospect for safe and effective CNV treatment. To enhance therapeutic efficacy and improve patient compliance, Yuan et al[109] developed nanowafer-loaded axitinib. As expected, axitinib nanowafers administered once a day were therapeutically twice as effective as axitinib delivered twice a day by topical eye drop therapy in treating CNV in a murine ocular burn model.

EGCG, the major active component of green tea, has been shown to inhibit angiogenesis via inhibition of vascular endothelial cell growth, thus exhibiting considerable potential in the field of inhibiting CNV.[120] Miyagawa et al[42] designed an eye drop formulation containing EGCG NPs for targeted therapy in CNV. The authors claimed that this eye drop formulation can effectively target corneal vessels and thereby inhibit chemical cauterized-induced CNV by a once-daily treatment. Other plant extracts, including celastrol, curcumin, kaempferol and naringenin, are hydrophobic molecules that limit their clinical use. In response, they were encapsulated in micelles,[43] NPs,[44] GNPs[45] and microemulsions[46] respectively to greatly improve their water solubility while still retaining their activities in CNV therapy.

The Wnt signaling pathway, which is an upstream signaling pathway regulating VEGF, plays an indispensable role in developmental and pathological ocular angiogenesis.[121,122] Inhibitors of Wnt signaling have displayed therapeutic

potential in treating Wnt pathway–dependent diseases. Zhong et al[63] constructed liposomes loaded with the Wnt/β-catenin pathway inhibitor XAV939 (XAV939 NPs). After being topically applied to alkali-burned corneas, XAV939 NPs enhanced corneal wound healing and suppressed CNV. Moreover, the expression of angiogenic and inflammatory-related genes was inhibited by XAV939 NPs.

TAK1 is an emerging therapeutic target for pathologic angiogenesis since it closely engages in several important angiogenic activities.[123] Wang et al[124] developed a novel eye drop formulation based on GNPs to encapsulate the selective TAK1 inhibitor 5Z-7-oxozeaenol to treat CNV in a rodent model. Topical administration of GNP-encapsulated 5Z-7-oxozeaenol led to significantly greater suppression of CNV in a mouse model than the free form of 5Z-7-oxozeaenol.

Gene Therapy Based on ONS

Various ONS have been investigated, and they offer considerable benefits in gene therapy for CNV (Table 2).

Among multiple targets of gene therapy in CNV, VEGF is the most common therapeutic target.[125] Antiangiogenic soluble VEGFR-1, also called sFlt-1, is essential for preserving the avascular ambit of the cornea. Normal human corneas strongly express sFlt-1 in the corneal epithelium and weakly in the corneal stroma close to the limbus.[7] Albumin NPs,[126] PLGA[127] and polymeric micelles[98] were able to deliver plasmid DNA encoding sFlt-1 or Flt23k (the VEGF-binding domains 2–3 of Flt-1) to the mouse cornea via intrastromal injection or subconjunctival injection. These strategies all showed prolonged gene expression with low cytotoxicity and significant inhibition of CNV in mice. VEGF-A is a key proangiogenic signal that can bind and activate VEGFR-2 to promote angiogenesis.[128] Qazi et al[129] loaded PLGA NPs

Table 2 Gene Therapy Based on ONS for CNV

ONS Type	Target Gene	Subjects	Models	Delivery	Dose	Results	Ref.
Lipoplexes	GA-binding protein	Mouse	Alkaline	Subconjunctival injection	2 µg in 20 µL after insult	Reduced CNV area by 20.3%	[133]
Lipofectine	VEGI	Rabbit	Suture	Subconjunctival injection	20 µL after insult	Reduced CNV by 69.8%	[132]
PLGA NPs	VEGF-A	Mouse	Alkaline	Intrastromal injection	2 µg plasmid/eye at 4 weeks after injury	Reduced CNV area by 43.0%	[129]
PLGA NPs	Flt23K	Mouse	Transplant	Subconjunctival injection	10 µL of plasmid (0.1 µg/µL) at day 0 and 4 weeks after transplant	Reduced CNV area by 71.0%	[127]
PLGA NPs	VLN	Mouse	Alkaline	Eye drops	20 µL,10 mg/mL at 1 hour after insult	ReducedCNV area by 50.0%	[138]
Albumin NPs	Flt23K	Mouse	Alkaline	Intrastromal injection	2 µg at 3 weeks before corneal injury	Reduced CNV area by 40.0%	[126]
rBPEI-NPs	NFκB	Mouse	Alkaline	Subconjunctival injection	10 µg of siRNA/eye at day 0, 3, 5 after insult	Reduced CNV area by 56.9%	[135]
Polymeric micelles	Flt-1	Mouse	Nylon suture	Subconjunctival injection	1 mg in 5 µL after insult	Reduced CNV area by 45.0%	[98]
Nanoemulsion	VEGFR-2	Mouse	Alkaline	Subconjunctival injection/Eye drops	10 µL after injury	Reduced CNV area by 89.0%/ 83.0%	[130]

Abbreviations: ONS, Ocular nanosystems; CNV, Corneal neovascularization; PLGA, Poly (lactide-co-glycoside); NPs, Nanoparticles; rBPEI, Reducible branched poly-ethylenimine; VEGI, Vascular endothelial cell growth inhibitor; VEGF-A, Vascular endothelial growth factor-A; Flt23k, The VEGF-binding domains 2–3 of Flt-1; VLN, Very low-density lipoprotein receptor N-terminal ectodomain; NFκB, Nuclear factor kappa B; VEGFR-2, Vascular endothelial growth factor receptor 2; siRNA, Small interfering RNA.

with a plasmid containing a short hairpin RNA (shRNA) expression cassette against VEGF-A and applied it to the mouse cornea via intrastromal injection. They claimed that this nanoscale therapeutic strategy can provide highly efficacious, sustainable, nontoxic regression of CNV. Similarly, ocular delivery of antisense oligonucleotide by cationic nanoemulsion directed at VEGFR-2 elicited a significant inhibition of CNV in mice.[130] Vascular endothelial cell growth inhibitor (VEGI) was reported to be a novel cytokine that can inhibit the proliferation of endothelial cells and angiogenesis.[131] Mediated by liposomes, VEGI cDNA was successfully delivered into all layers of the cornea and achieved 13.8 mm^2 less rabbit CNV after a silk suture was placed, which was unlike the results of the controls.[132]

GA-binding protein (GABP) is a transcription factor that regulates the expression of VEGF.[6] Yoon et al[133] used a lipid-based vector to deliver a plasmid DNA-encoding GABP to the mouse cornea via subconjunctival injection. This approach was shown to decrease VEGF gene expression and delay CNV for up to 2 weeks in a mouse model of deliberate corneal injury.

Another transcription factor, nuclear factor kappa B (NFκB), was reported to regulate the expression of various genes implicated in inflammation and angiogenesis.[134] Han et al[135] selected siRNA targeting NFκB p50 and loaded it within reducible branched polyethylenimine nanoparticles (rBPEI-NPs) to develop a new siRNA carrier as a hope for CNV therapy. This novel siRNA carrier achieved a desirable distribution of therapeutic siRNA inside the diseased CNV region and showed successful gene knockdown of angiogenesis without significant cytotoxicity in rat models of corneal alkaline injury.

Previous studies have reported that both very-low-density lipoprotein receptor (VLDLR) and VLDLR N-terminal ectodomain (VLN) have an inhibitory effect on the Wnt signaling pathway.[136,137] Wang et al[138] encapsulated an expression plasmid of VLN with PLGA NPs and applied it to treat mouse CNV induced by alkali burns. They demonstrated that this nanoscale therapeutic strategy can suppress Wnt signaling and ameliorate CNV. The VLN-NP-treated group developed fewer vessels in the cornea with alkali burns than the controls.

ONS as Therapeutic Agents

Some ONS, such as inorganic NPs, are rarely used to deliver any therapeutic agents but are themselves the therapeutic agents.[139–141] Cho et al[142] applied AuNPs in a mouse model and found that topical administration of AuNPs significantly reduced the development of CNV by inhibiting the ERK pathway. Since inflammation is a central pathological process in the formation of CNV, Luo et al[80] developed dual-functional (antibacterial and antiangiogenic) gelatin-capped AgNPs and demonstrated that intrastromal administration of highly biocompatible gelatin-capped AgNPs alleviates S. aureus-induced bacterial keratitis in rabbit eyes and bacterial infection-induced CNV. Zheng et al[143] synthesized CeNPs with antioxidant efficacy and attempted to use their antioxidant capacity for the treatment of inflammation-associated CNV. The synthesized CeNPs showed good biocompatibility and were capable of controlling inflammation and neovascularization by blocking oxidative stress. Topical SiNPs can also effectively decrease the extent of CNV.[144] Tang et al[145] prepared c(RGDyC)-tagged silicon nanoparticles (SiNPs-RGD), a theranostic agent made of peptide-functionalized SiNPs that is suitable for simultaneous ocular neovascularization imaging and therapy. Their results indicated that intravenous injection of SiNPs-RGD can selectively detect angiogenic blood vessels in vivo as well as inhibit alkali burn-induced CNV.

Conclusion and Future Perspectives

The novel ONS based on nanotechnology represent a new avenue for the treatment of CNV. Compared to traditional drug delivery, ONS can not only optimize ocular penetration and bioavailability, but also prolong drug retention time, thereby achieving sustained delivery and controlled therapeutic release with minimal toxicity and side effects (Table 3). Some inorganic nanomaterials, such as AuNPs,[142] AgNPs,[80] CeNPs[143] and carbon-based nanomaterials,[146] are themselves the antiangiogenic nanoagents for antiangiogenic applications. In the field of gene therapy for CNV, various ONS have been utilized as biocompatible nanocarriers to achieve high efficiency of gene transfection and long-term gene expression. Notably, ONS for CNV therapy are still in the preliminary research stage; thus, appropriately designed clinical trials for various ONS in the treatment of CNV are needed in the future. Several challenges remain to be addressed for translation from the bench into clinical products, including selection of various nanocarrier forms, safety control of potential toxicity associated with ONS and large-scale production of ONS.

Table 3 Features of ONS for Drug Delivery in CNV Therapy

Features	Ref
Improve bioavailability, solubility and penetration of drugs into cornea	[39,40,117–119]
Sustained release in a controlled manner	[39,111,114,115]
Better corneal retention time	[42,45,63,119]
Reduced drug-associated ocular irritation and toxicity	[41,114,115,117–119]
Nano-formulation can control drug activity by releasing only at the desirable ocular site with prolonged therapeutic effects to reduce frequent doses	[109,112,114]
Great drug efficacy in reduced CNV	[64,109,114,124]

Abbreviations: ONS, Ocular nanosystems; CNV, Corneal neovascularization.

Nanocarriers are designed to overcome the limitations associated with current CNV therapy and ensure targeted and controlled drug delivery.[147,148] Therefore, therapeutic drugs are of top priority in nanoformulations and full consideration about their properties should be given when selecting nanocarrier forms. For instance, some antiangiogenic drugs are hydrophilic, while others are hydrophobic; some nanocarrier forms can load both hydrophilic and hydrophobic molecules, while others can deliver only either hydrophilic or hydrophobic molecules. Hence, it is indispensable to carefully consider the characteristics of each antiangiogenic drug to select the most suitable nanocarrier forms to optimize the antiangiogenic efficacy of the tailored nanoformulation to meet specific clinical needs.[20]

Although various efforts have been made to understand the toxicity of ONS to eyeball tissues, preclinical toxicology data of animal models are usually used, and human clinical trials are lacking.[11,12] With the further development of ONS for drug delivery and gene therapy for CNV, appropriately designed clinical trials for various ONS should be conducted in the future. Nanotoxicity depends heavily on a variety of properties, such as material composition, concentration, biodistribution, size, shape, surface charge, attached chemical groups and zeta potential.[149,150] For example, positively charged biopolymers containing nanoformulations prolong the drug retention time at the ocular surface.[20] However, prolonged residence time might also potentially provoke corneal toxicity that necessitates further investigation. In addition, nanoformulations that have a surfactant concentration that is too high could cause corneal damage.[151] In some cases, the presence of surfactants may cause a sticky sensation and blurred vision upon instillation, hence impeding patient compliance.[152] Most inorganic nanoparticles are relatively hard to degrade and eliminate from the human body, which may cause excess retention in living organisms and harmful effects such as inflammation and tissue damage.[70] Therefore, it is essential to carefully evaluate the strengths and limitations of each formulation to maximize the therapeutic effects of active drugs while minimizing the potential systemic or local toxic side effects. Continuous optimization with appropriate modification and functionalization of the formulation would be beneficial for clinical translation.[20] Special considerations should also be given to the techniques for sterilizing the ONS for CNV therapy, such as aseptic processing/sterile filtration or terminal sterilization by autoclaving.[113] Furthermore, regulatory bodies need to establish guidelines to ensure the safety and quality of various ONS formulations for CNV therapy.[153]

The formulations of ONS for CNV therapy are versatile. The unique customizable feature of each nanoformulation may result in a lack of standardized protocols for large-scale production. In addition, the production of ONS usually involves a multistep and complex process that includes multiple components. It is difficult to achieve a robust process to maintain repeatability. Moreover, the complex production process also greatly increases production costs.[11] In response, recent advances in technology, such as microfluidic, high-pressure homogenization, particle replication in nonwetting template and hydrogel template methods, have been investigated and have provided hope for large-scale production.[154] In the future, cost-effective technology also needs to be developed to scale up the production of ONS designed for CNV therapy.

Taking all aspects into account, more meticulous work is needed before ONS-based products for CNV therapy are successfully on the market. Nevertheless, various preclinical studies conducted to date have demonstrated promising

results of the use of ONS in the treatment of CNV. We believe that, in the future, ONS will play a more significant role in the treatment of CNV in different stages, with the hope of circumventing the common limitations of currently available treatment strategies in the clinic.

Abbreviations

ONS, Ocular nanosystems; CNV, Corneal neovascularization; VEGF, Vascular endothelial growth factor; PDT, Photodynamic therapy; FND, Fine-needle diathermy; VECs, Vascular endothelial cells; VEGFR, Vascular endothelial growth factor receptor; MMPs, matrix metalloproteinases; bFGF, basic fibroblast growth factor; DEX, Dexamethasone; NSAIDs, Nonsteroidal anti-inflammatory drugs; CsA, Cyclosporine A; BEV, Bevacizumab; EGCG, Epigallocatechin gallate; siRNA, Small interfering RNA; NPs, Nanoparticles; PLGA, Poly(lactide-co-glycoside); PLA, Poly(lactide); PCL, Poly(ε-caprolactone); HA, Hyaluronic acid; PEG, Poly(ethylene glycol); GNPs, Gelatin nanoparticles; AuNPs, Gold nanoparticles; AgNPs, Silver nanoparticles; SiNPs, Silica nanoparticles; CeNPs, Cerium oxide nanoparticles; PAMAM, Poly(amidoamine); DSP, Dexamethasone sodium phosphate; MPEG-hexPLA, Methoxy poly(ethylene glycol)-hexylsubstituted poly(lactides); MSNs, Mesoporous silica nanoparticles; HSA, Human serum albumin; MPEG-PCL, Methoxy poly(ethylene glycol)-poly(ε-caprolactone); shRNA, Short hairpin RNA; VEGI, Vascular endothelial cell growth inhibitor; GABP, GA-binding protein; NFκB, Nuclear factor kappa B; rBPEI-NPs, Reducible branched poly-ethylenimine nanoparticles; VLDLR, Very-low-density lipoprotein receptor; VLN, VLDLR N-terminal ectodomain; SiNPs-RGD, c(RGDyC)-tagged silicon nanoparticles.

Acknowledgments

Funding

Disclosure

The authors declare that they have no competing interests.

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