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Nanoparticle-mediated corneal neovascularization treatments: Toward new generation of drug delivery systems

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ABSTRACT

Corneal neovascularization (CNV) is one of the major factors for vision impairment and blindness worldwide. The current treatment for CNV focuses primarily on topical eyedrops of glucocorticoids, non-steroidal anti-inflammatory drugs, electro-coagulation and laser photo-coagulation. Unfortunately, coagulation-based treatment is restricted by corneal hemorrhage and iris atrophy. And drug treatments have limited therapeutic effects and a short duration of action. Nanoparticle-based drug delivery systems are widely applied due to their improved pharmacokinetics, optimized drug targeting and enhanced biocompatibility. In this article, we provide a comprehensive and systematic overview of the CNV nanodrug system, highlighting some of the recent advances in nanodrug design, preparation, and functional modification. Moreover, we discuss the challenges in the clinical translation and potential risks in CNV treatment. A greater effort is needed for the potential applications of nanotechnology in the field of ophthalmology.

1. Introduction

Corneal neovascularization (CNV) is an ocular disease that blood vessels spread over the avascular cornea due to alkali burn, virus infection, graft rejection and limbal stem cell deficiency, and finally resulting in corneal opacity, reduced vision and even blindness [1]. Therefore, effective, non-invasive, or minimally invasive methods of inhibiting the occurrence and development of CNV have continuously been an urgent problem in ophthalmology.

The current treatments for CNV include surgery and drug therapy. In surgical treatment, argon laser and Nd:YAG lasers are used to block blood vessels immediately through photothermal damage [2], while common complications such as corneal hemorrhage, corneal thinning and iris atrophy have limited their applications. Among various types of drug therapies, topical administration of corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) remain top priority. The study of vascular endothelial growth factor (VEGF) as a therapeutic agent for inhibiting CNV is very plausible [3]. Anti-VEGF drugs like bevacizumab and small interfering RNA (siRNA) have been applied [4]. Additionally, NSAID usage may lead to cataracts and glaucoma. Similarly, superinfections and anti-VEGF drugs do not cure existing blood vessels, thus requiring multiple injections. Moreover, other shortcomings such as low levels of drug concentration in the targeted area, rapid removal of tears and blockage of the corneal barriers have also limited the effectiveness of drug therapy [5]. Furthermore, some Chinese traditional medical methods such as herbal extracts, including tripterine [6], osthole [7] and curcumin [8] as well as acupuncture also showed therapeutic effects for CNV to a certain extent [9].

To ameliorate the bioavailability of ocular medicine, researchers have constructed a variety of nano delivery structures, like liposomes, non-viral vectors, *etc*. Recently, nanoparticle-based drug delivery systems are emerging for CNV treatment [10,11]. Nanomaterials can not only enhance biological compatibility but also increase drug accumulation in neovascularized areas [12]. Among

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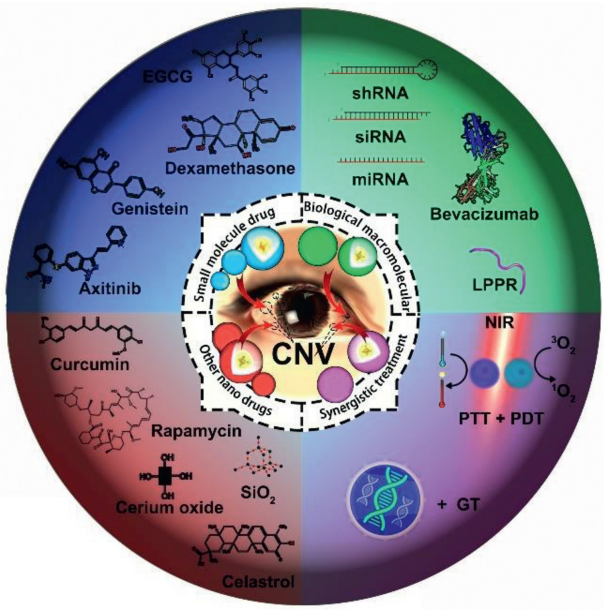
Table 1
Nanocarriers to deliver anti CNV agents.

Classification	Drugs	Nanocarriers	Particle size (nm)	Improvement strategy	Models	Route	Refs.
Small molecule drug loading							
Anti-VEGF drugs	EGCG	HA-RGD	158.10 ± 11.06	RGD polypeptide targeting	Chemical cauterization-induced CNV mice	Topical	[22]
	Axitinib	MPEG-PCL	53.01 ± 0.48	Improved hydrophilicity	Alkali-induced CNV mice	Topical	[28]
	Cabozantinib	Lys (NH_2)-Phe	106.7	Cationic polypeptide micelles electrostatic adhesion			[31]
Glucocorticoid	Dexamethasone	PLGA	200	Zn^{2+} bridges PLGA-COOH and DEX, surface modification biological adhesive boric acid	Suture-induced CNV in rats	SCT	[36,37]
		Boric acid	~80				
Biological macromolecular loading							
Anti-VEGF antibody/peptide	Avastin	PLGA	90	Mesoporous silica nanoparticles or PLGA encapsulation	Alkali-induced CNV mice	SCT	[40,42]
		Silica	140 ± 18				
	Flt1 peptide, Genistein	HA	172.0 ± 18.7	Flt1 peptide-HA conjugate micelles encapsulating genistein	Silver nitrate cauterization-induced CNV in rats		[32]
	LPR	Polypeptide amphiphilic nanofiber	10-20	Self-assembled peptide amphiphilic nanofiber system	Suture-induced CNV in rats		[45]
	Flt23k	PLGA-RGD	270.2	RGD polypeptide targeting, PLGA- encapsulation			[47]
Gene treatment delivery system	NF-κB-siRNA	rBPEI-NPs	164.5 ± 38.2	PEI electrostatic adsorption	Chemical cauterization-induced CNV in rats	SCT	[61]
	shRNA	SLN	190 ± 3	Cationic SLN adhesion, PLGA-encapsulation	HCE-2 cells		[63, 65]
	miR-132	Polymer H3K4b	150	polymer H3K4b encapsulation	HSV-infected mice	SCT	[67]
Other nano drugs							
		CeNPs	20		Alkali-induced CNV in rats/mouse	Topical	[70,72-74,81]
		SiNPs	30.1 ± 5.6				
		AuNPs	20				
		G-Ag NPs	13.6	Gelatin encapsulation			
		BSA-GO	~70	Serum albumin-capped GO			
Synergistic treatment	Bevacizumab	R-s-ICG	143.2	Verteporfin-based PDT, PT, GT	Suture-induced CNV in rats	Topical and SCT	[88–90]

various routes for ocular administration, topical administration is the most widely used modality. While eyedrops can be limited by tear film and corneal barriers [13]. Nanodrug systems that are biocompatible, permeable, and stable can improve the effectiveness of eyedrops and achieve efficient enrichment in the target tissue [14,15]. To achieve ideal therapeutic effects, researchers often use poly(lactide-glycolide) (PLGA), hyaluronic acid (HA) and other nanomaterials as drug carriers (Table 1). In this article, we briefly review the recent progress of nano-loaded system for CNV treatment (Scheme 1): (i) small molecule drug; (ii) biological macromolecular; (iii) other nano drugs; (iv) synergistic treatment. Finally, we prospect further application of nano drugs in CNV treatment.



Scheme 1. The graphic illustration for nanoparticles-mediated CNV treatments.

2. Small molecule drug loading

2.1. Anti-VEGF drugs

Under normal conditions, ocular vascular homeostasis is regulated by two systems: angiogenic stimulators and angiogenic inhibitors. In pathological conditions, the balance between anti-angiogenic and pro-angiogenic effects is disrupted, leading to the formation of blood vessels [16]. Hence, therapeutic strategies targeting VEGF and its receptor are extremely important in CNV treatment [17,18]. Up to now, many anti-VEGF agents have been applied

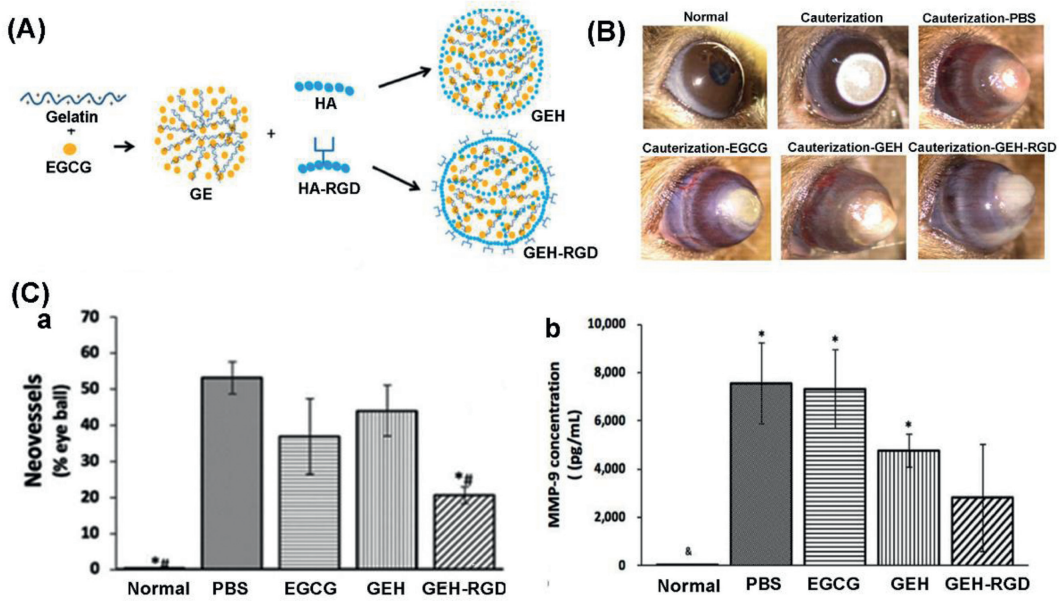


Fig. 1. (A) Synthesis of EGCG loaded GEH-RGD nanoparticles. (B) EGCG nanoparticles inhibit neovascularization in chemically cauterized CNV. Typical images of normal cornea burned cornea, and cornea treated with PBS, EGCG, GEH, or GEH-RGD on day 7 after chemical burn. (C) The corneal vascular area (a) and MMP-9 (b) of all treated groups were quantified. Reproduced with permission [22]. Copyright 2020, MDPI.

in CNV treatments, such as triamcinolone acetonide [19], fasudil [20].

Epigallocatechin-3-gallate (EGCG) is a catechin monomer isolated from tea with an anti-angiogenesis effect [21]. As shown in Fig. 1A, Miyagawa *et al.* contrived gelatin-EGCG nanoparticles (GE NPs) and then modified them by the prepared arginine-glycine aspartic acid (RGD) coupled HA (HA-RGD), constructing the HA-RGD-modified GE NPs (GEH-RGD) [22]. The substance HA, an ingredient of corneal stroma, plays a critical role in corneal cell development and growth [23], and RGD has been confirmed to target newly formed blood vessels [24]. To assess the anti-angiogenic effect of GEH-RGD NPs, a chemical burn-induced CNV mouse model was constructed. As shown in Figs. 1B and C-a, dense endophytic blood vessels surrounding the entire cornea were observed in both PBS and EGCG groups on day 7. In addition, the GEH-RGD group had better corneal transparency and minimal neovascularization compared with other groups. Then, the expression of matrix metalloproteinase 9 (MMP-9) and VEGF protein in the cornea were detected by ELISA. With regard to the PBS group, VEGF levels were significantly reduced in the GEH-RGD group. Meanwhile, the level of MMP-9 in the GEH-RGD NPs group was also significantly decreased (Fig. 1C-b). Thus, the analysis of MMP-9 and VEGF expressions indicated that GEH-RGD may treat CNV through the inhibition of VEGF and MMP-9 in chemically burned corneal tissues.

Tyrosine kinase inhibitors (TKIs) are a set of enzymes that restrain tyrosine kinase activity containing vascular endothelial growth factor receptor 1 (VEGFR1), VEGFR2, VEGFR3 and platelet-derived growth factor receptors (PDGFR) [25]. Among them, VEGFR2-targeted TKIs are widely studied due to their ability to block all sorts of signaling molecules in downstream pathways and inhibit cell proliferation and differentiation [26]. Axitinib, a hydrophobic drug, is the second generation of tyrosine kinase targeting inhibitors with specific pharmacological targets and molecular mechanisms. Yuan *et al.* showed that axitinib nanocrystals prepared by engineering methods could enhance therapeutic effects in CNV treatment [27]. Shi *et al.* modified axitinib with amphiphilic copolymer polyethylene glycol-polycaprolactone (MPEG-PCL) to improve the liquid dispersity of hydrophobic axitinib [28]. Then, they demonstrated the therapeutic effect in alkali burn-induced CNV rat model by reducing the infiltration of inflammatory cells into corneal tissue, as well as the number and lumen size of neovascularization, exhibiting an anti-angiogenesis effect without significant tissue toxicity.

Cabozantinib, also known as XL184, is a multi-targeted small-molecule TKIs that has been applied to remedy various solid tumors by blocking VEGF signaling pathway [29,30]. The ability to inhibit the development of vascular endothelial cells also enables cabozantinib to be a candidate drug for CNV treatments. To reduce its systemic toxicity, Han *et al.* constructed *N*-carboxyanhydride ring-opening polymerization (NCA-ROP) and then based on cationic amphiphilic polypeptide Lys (NH_2)-Phe to encapsulate lipophilic cabozantinib into self-assembled micelles (Cabo-NPs) [31]. Cationic Cabo-NPs could bind with corneal surface mucins by electrostatic action to avoid rapid clearance and prolong the retention time in the eye, thereby enhancing its bioavailability (Fig. 2A). Afterwards, they used an alkali burn-induced CNV model to evaluate the efficacy of Cabo-NPs. Cabo-NPs showed stronger anti-CNV effects than other groups *via* strong inhibition of pro-inflammatory factors and pro-angiogenic factors (Fig. 2B-a). The amount of angiogenesis reached the highest level on day 14. The saline group exhibited the most severe CNV accompanied by turbidity and edema. There was no significant difference in the length of blood vessels and neovascularized area between saline group and cabozantinib group, indicating that cabozantinib alone had no significant inhibitory effect on CNV. In contrast, vessel length and neovascularized area were significantly reduced in Cabo-NP-treated mice (Figs. 2B-b&c). Meanwhile, the positive control group of Dexp, showed similar clinical examination score as Cabo-NPs (Fig. 2B-d), indicating the potential value of Cabo-NPs for further clinical application.

Genistein, another TKIs, can regulate the occurrence and development of CNV. To deliver genistein, the antagonistic targeting micellar delivery system was prepared by conjugating Flt1 peptide on HA (Flt1 peptide-HA) [32]. In a further step, genistein was encapsulated in Flt1 peptide-HA. The assembled micellar particles were spherical particles with 172.0 ± 18.7 nm in size. The CNV inhibition effect was quantitatively analyzed by comparing all the six groups. Thus, the constructed genistein/Flt1 peptide-

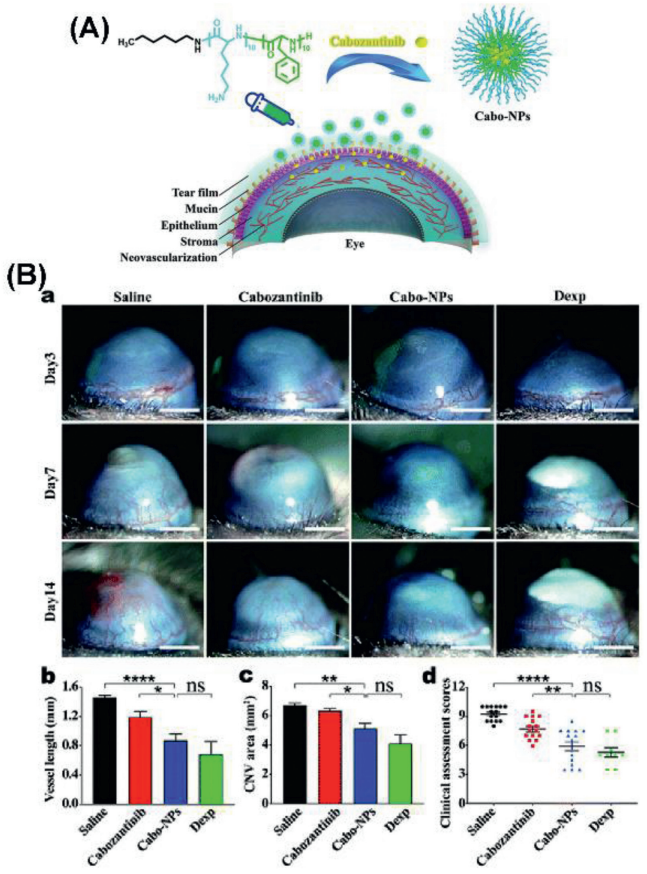


Fig. 2. (A) Schematic diagram of preparation and CNV inhibition of mucoadhesive Cabo-NPs. (B) Anterior eye segment photos represent morphological differences caused by different treatments after alkali burn in mice (a); after being treated for 14 days, the vessel length (b) and neovascularized (c) were measured; (d) clinical evaluation score reflecting the therapeutic effect of corneal alkali burn. Reproduced with permission [31]. Copyright 2020, The Royal Society of Chemistry.

HA micelle can also be a potential treatment in other ocular diseases.

2.2. Glucocorticoid

For corneas, the topical application of corticosteroids has become a standard anti-inflammatory treatment [33]. The dexamethasone sodium phosphate eyedrops, part of the corticosteroid family, have been used in clinical trials for a long time. Dexamethasone (DEX) is a synthetic glucocorticoid hormone that can be used to treat inflammation and injury. DEX can effectively frustrate the production of pro-inflammatory cytokines, and decrease the proliferation and migration of vascular endothelial cells, thereby reducing the inflammatory response and injury yielded by CNV [34]. However, the side effects of DEX, such as secondary corneal infections, glaucoma, ocular hypertension, and cataracts, have limited its use [35].

Wang *et al.* prepared biodegradable nanoparticles by combining carboxyl terminated poly(lactic-*co*-glycolic acid) (PLGA-COOH), with dexamethasone sodium phosphate (DSP) to prepare DSP-Zn-NP (Fig. 3A-a) [36]. DSP-Zn-NP was a 200 nm spherical particle with uniform distribution (Fig. 3A-b). The release rate of DSP from DSP-Zn-NP implied the strong binding through zinc ion (Fig. 3A-c). After subconjunctival (SCT) injection of DSP-Zn-NP, the NPs could release DSP in the anterior chamber, which significantly improved drug retention time to almost 14 days as well as increased drug concentration (Fig. 3B). DSP-Zn-NP could enhance CNV inhibition without increasing intraocular pressure or producing harmful substances in the injection areas. On day 14, the corneal vascular length and neovascularization area were quantified, and it was found that the quantitative value of the DSP-Zn-NP group was the smallest, which also proved the anti-CNV effect of DSP-Zn-NP. Thus, the use of DSP-Zn-NP for SCT treatment may be an expected method to treat CNV with the enhancement of patient compliance and the reduction of graft rejection.

In a further study, Zhang *et al.* prepared a nanoscale therapeutic platform *via* boric acid chemical and loaded it with DEX (Fig. 4A) [37]. With the help of surface-modified boric acid, the DEX-loaded NPs could adhere to the corneal surface and then permeate into the corneal stroma (Fig. 4B). In an injury-induced CNV model, the Nile red labeled NPs were studied, and compared with free form, more Nile red was coated in the nanoparticles and distributed on the corneal epithelium, some of which even penetrated the stromal layer and entered the endothelial layer. Furthermore, most of the Nile red (red) signals overlapped well with those of the nanoparticles (green), suggesting that the drug entered the cornea *via* the action of boric acid-derived nanoparticles. Finally, the DEX-loaded NPs had the smallest neovascularized area in CNV treatment and improved the efficiency (Figs. 4C and D). The bio-adhesive nanomaterials showed their promising candidate for the effective treatment of ocular diseases. Based on the studies above, it is suggested that we can use PLGA, HA and other commonly used nano drug carriers to wrap small molecule drugs with low hydrophilicity and high toxicity.

3. Biological macromolecular loading

3.1. Anti- VEGF antibody/peptide

Anti-VEGF antibodies in clinical trials and animal models have been confirmed to frustrate CNV [38]. Avastin, also known as bevacizumab, is a recombinant humanized monoclonal antibody. It can bind to VEGF in order to prevent VEGF from binding to VEGFR. Currently, it is widely used to treat renal cell carcinoma, glioblastoma, choroidal neovascularization, retinal vein occlusion and proliferative diabetic retinopathy (PDR). While frequent injections are required due to the short half-life of bevacizumab, which has limited its application and efficiency [39]. To further modify bevacizumab, Zhang *et al.* found that bevacizumab modified with PLGA exhibited enhanced therapeutic effects and bioavailability with less toxicity to normal tissues [40].

Various strategies have been proposed to keep the drug on the ocular surface for a longer time and be better absorbed, such as the use of *in situ* gel excipients and osmotic promoters [41]. Sun *et al.* used the nano-casting method to coat bevacizumab nanoparticles with mesoporous silica nanoparticles (MSNs), and then conducted Fourier transform infrared, transmission electron microscopy and other ways to estimate the anti-angiogenesis effect of MSN-Bevacizumab NPs *in vitro* and *in vivo* [42]. The researchers found that MSN extended the retention time of bevacizumab in the eye and maintained drug concentration for a longer time. In addition, it was more effective than bevacizumab alone in limiting VEGF-induced endothelial cell proliferation, migration and neovascularization. These nanoparticles could serve as a continuous drug delivery system and a latent carrier for CNV treatment.

Previously, the LPPR peptide sequence has been shown to strongly inhibit VEGF activity by specific binding to VEGFR1 *via* phage display technology [43]. In addition, similar peptide sequence D (LPR) has also shown promising results in the treatment of retinopathy of prematurity [44]. Based on this, Senturk *et al.* made the peptide sequence LPR epitope on the polypeptide amphiphilic nanofiber system and increased the anti-angiogenesis ability of the epitope to form LPPR-PA nanofiber, which can im-

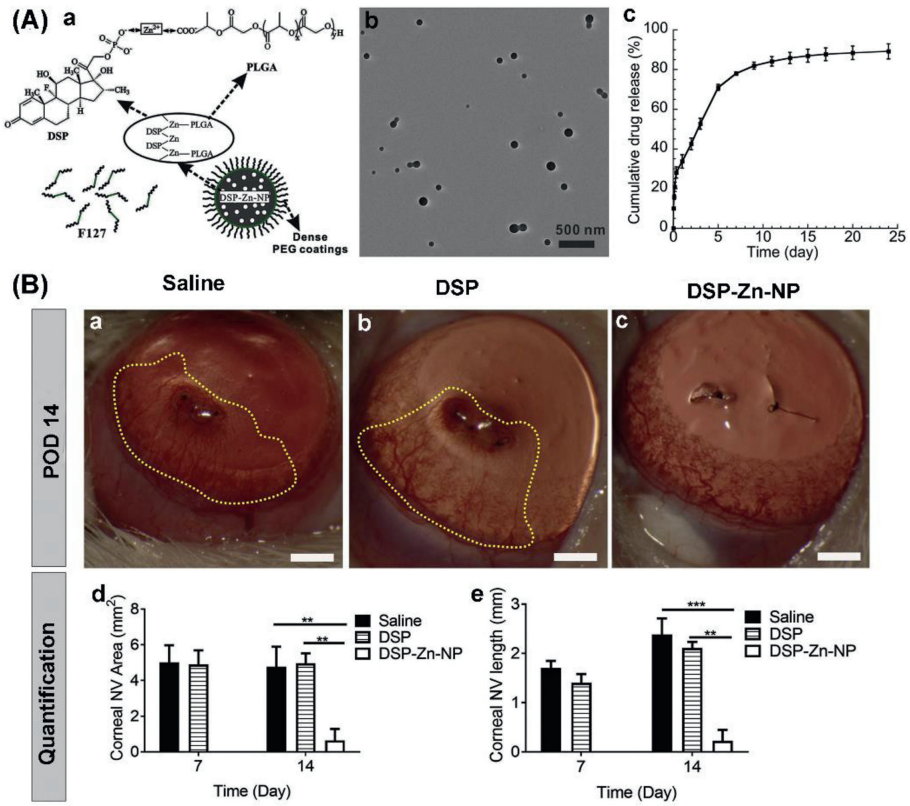


Fig. 3. (A) Preparation scheme of DSP-Zn-NP (a), TEM image of DSP-Zn-NP (b) and cumulative drug release (c). (B) Typical slit-lamp images of rat cornea after SCT treatment at 7 and 14 days (a-c), quantize CNV area (d) and length (e). Reproduced with permission [36]. Copyright 2019, Elsevier Inc.

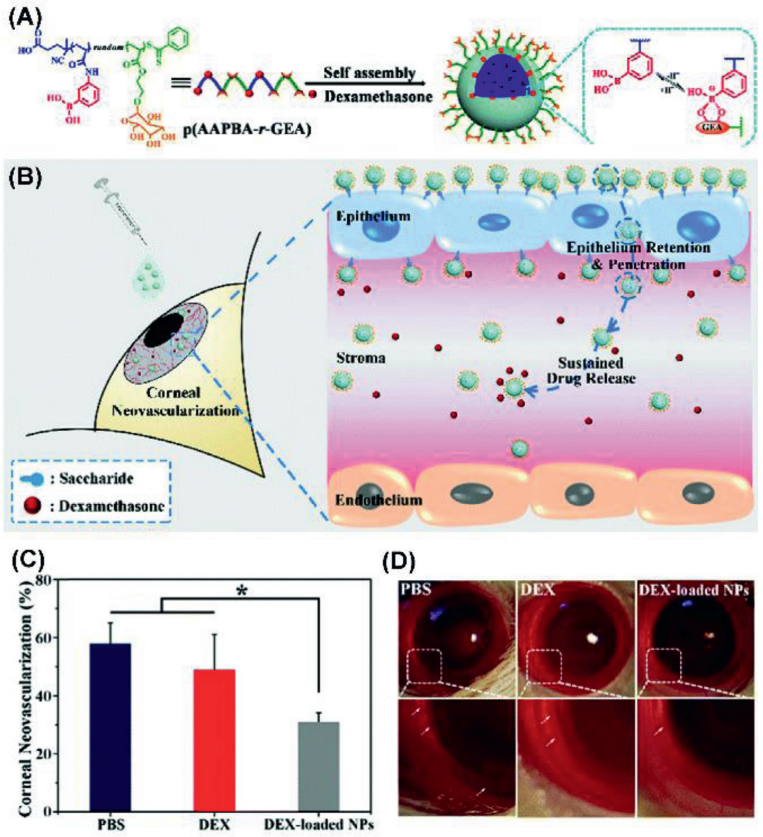


Fig. 4. (A) Synthesis of glycopolymers nanoparticles. (B) Schematic diagram of CNV inhibition by bio-adhesion, epithelial cell penetration, and drug release simulating glycyrrhiza bio-adhesion nanoagents. (C) Statistical analysis of CNV after 7 days of treatment. (D) Typical images of CNV 7 days after treatment. Reproduced with permission [37]. Copyright 2021, The Royal Society of Chemistry.

prove its prevention efficiency of neovascularization [45]. The results implied that its ability to inhibit endothelial cell proliferation, tubular formation and migration was better than soluble LPPR peptide *in vitro*. Moreover, its anti-angiogenesis effect was similar to the commercial drug bevacizumab, which was superior to the soluble polypeptide preparation and may become a potential way to treat CNV.

Among VEGF inhibition approaches, nanoparticles conveying anti-VEGF effects are also a potential strategy for intracellular targeting of VEGF. The anti-VEGF receptor Flt23k is a recombinant vector of the VEGFR1 binding domain and connects to the endoplasmic reticulum (ER) keeping signal sequence lysine-aspartic acid-glutamate-leucine (KDEL) [46]. Flt23k receptor can bind with VEGF in cells with high affinity and isolates it from the endoplasmic reticulum, thus hindering VEGF autocrine cycle and secretion. Cho *et al.* confirmed that nanoparticles conveying anti-VEGF receptor Flt23k could enhance the survival rate of corneal transplantation in mice, as well be endowed with the abilities of anti-neovascularization, reduction of lymphangiogenesis in penetrating synergistic effect and corneal transplantation with steroid therapy [47].

3.2. Gene treatment delivery system

Gene therapy is an innovative approach to treat hereditary or acquired corneal diseases [48]. In fact, the adeno-associated virus (AAV) in viral vectors has enjoyed great popularity in corneal gene therapy [49]. The tissue-selective gonadal-associated virus (AAV5) was utilized to deliver core proteoglycan (Decorin, DCN) gene in rabbit corneas, which could inhibit the synthesis of VEGF and regulate angiogenesis [50]. Mohan *et al.* examined the gene therapy effect of AAV5-DCN over 6 months and showed that locally tissue-targeted AAV5-DCN were safe in the treatment of corneal fibrosis and neovascularization [51]. Other target genes have also been investigated using AAV, including VEGFR1, PEDF, MMP-9, *etc.* [52–54].

RNA interference is a promising gene silencing technique to inhibit CNV [55]. siRNA, a synthetic double-stranded small RNA sequence, can target genes specifically and silence therapeutic targets *via* RNA-induced silencing complex (RISC) [56]. Minimal toxicity is the primary requirement for a siRNA delivery system to treat CNV [57]. Among various gene therapy vectors, researchers prefer viral vectors [58]. While some potential problems like toxicity, immune and inflammatory responses prevent viral vectors from being utilized for diseases like ophthalmology. Thus, non-viral vectors are a potential strategy for virus-mediated gene delivery systems [59].

Among diverse siRNA carriers, polyethyleneimine (PEI) is considered to be a latent gene carrier in consideration of its functions to interact electrically with negatively charged siRNA and condense it into nanoparticles [60]. Accordingly, Han *et al.* synthesized a siRNA delivery system based on reducible branched-polyethylene imine (rBPEI) and applied it in CNV treatment (Fig. 5A) [61]. After SCT injection of siRNA-rBPEI-NPs, the drug was accumulated in vascular endothelial cells, and the -S-S- bond was reduced by over-expressed glutathione (GSH), thus inducing the depolymerization of the nanoparticles and the release of siRNA (Fig. 5B). And the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) was studied due to its important role in VEGFR3 expression. The degree of neovascularization was quantified by fluorescence intensity normalization (Figs. 5C and D). The results showed that NF-κB-siRNA-rBPEI-NPs had the strongest anti-angiogenesis effect, which was significantly higher than that of naked NF-κB-siRNA. The result implied that siRNA encapsulated in rBPEI could effectively retain in corneal tissues for a long time, thus ensuring the therapeutic effect of siRNA in the targeted area.

Short hairpin RNA (shRNA), another interfering RNA (RNAi) molecule, can downregulate the expression of target genes. shRNA provides a more attractive alternative to siRNAs in that it could minimize or avoid non-specific immune activation, which is a common side effect of siRNAs [62]. Additionally, cationic liposome nanoparticles (SLN) can interact with negatively charged mucus on the ocular surface after local administration, thus improving drug retention time and promoting penetration ability *via* endocytosis of corneal epithelial cells. Torrecilla *et al.* prepared a shRNA delivery system based on SLN by solvent emulsification and evaporation technology. The protamine in the system aquired a high concentration of genetic material, which could improve the transfection efficiency of non-viral vectors, thus protecting them from degradation and facilitating their entry into the nucleus [63]. The vector could reduce the cellular synthesis of MMP-9 protein by about 30%. A suitable vector was selected by comparing agarose gel results, total protein and percentage expression of HCE-2 transfected cells. It was verified experimentally that the addition of a carrier could reduce the migration of HCE-2 cells due to the inhibition of MMP-9 production, which degraded type IV collagen and gelatin substrate [64]. Therefore, the reduction of MMP-9 level could lead to the reduction of gelatin degradation ability. This strategy of designing lipid nanoparticles may be useful in the clinical treatment of corneal diseases in which inflammatory cytokines and MMP-9 activity are elevated.

To reverse CNV after alkali injury, Qazi *et al.* loaded plasmids containing anti-VEGF-A shRNA expression boxes into PLGA NPs, forming a sustainable and enhanced anti-angiogenesis method [65]. The nanoparticles with 200 nm in size, showed significant retention in periocular space for up to two months and a sustainable anti-angiogenic effect. They expected that PLGA NPs in the matrix would be cleared slowly based on polymer degradation time in order to treat CNV more durably and robustly than plasmids alone. Moreover, dose effects should also be considered once performed on primates and humans.

MicroRNAs (miRNAs) are small molecules that regulate gene expression at the post-transcriptional level *via* mediating mRNA degradation or inhibiting its transcription. miR-132 is a highly conserved miRNA with a mature sequence length of 22 bp, which is cleaved from the precursor sequence of miR-132 with a length of 66 bp [66]. Furthermore, miR-132 can be induced by growth factors such as VEGF in endothelial cells. Mulik *et al.* found low miR-132 levels in the corneas of IL-17 receptor gene knockout mice and the silence of miR-132 *in vivo* gave rise to CNV reduction *via* providing about 150 nm anti-miR-132 nanoparticles to the mice [67]. Thus, manipulation of miRNA expression is likely to be a latent therapeutic strategy to frustrate CNV progression.

Compared with small molecule drugs, the effect of biological macromolecular drugs by conjunctival injection is more significant, but there are risks of corneal epithelial damage and other long-term drug use. It is expected that researchers can find some nano-drug carriers in the future to modify the drugs, so that the eye drops of macromolecule drugs can achieve good therapeutic effects.

4. Other nano drugs

Reactive oxygen species (ROS) play a pivotal role in the pathogenesis of ocular diseases. Blocking oxidative stress *via* reducing ROS levels is an underlying therapeutic strategy for the treatment of ocular diseases, such as inflammation and CNV [68]. So far, multiple anti-oxidant drugs have been used like dexamethasone and bevacizumab. Cerium oxide nanoparticles (CeNPs) are nanocrystals derived from cerium, which have been studied for their high efficiency in ROS scavenging [69]. In a previous study, Zheng *et al.* contrived three types of CeNPs, characterized these

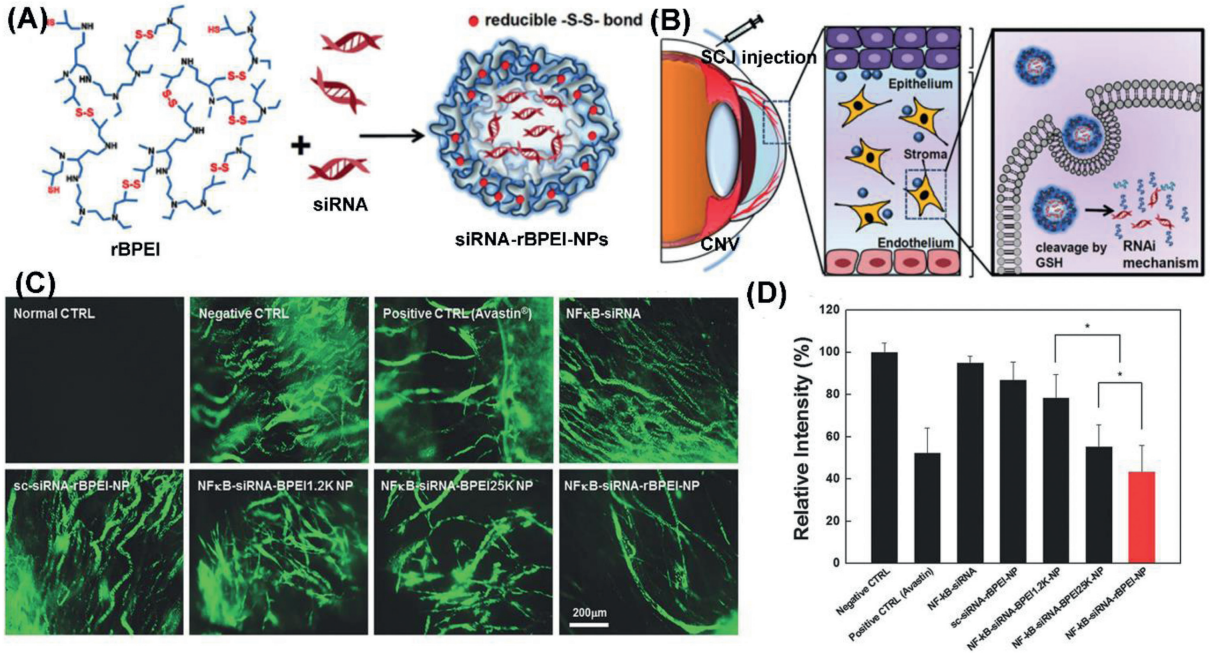


Fig. 5. (A) Preparation process of siRNA-rBPEI-NPs. (B) Schematic diagram of siRNA-rBPEI-NPs treatment of CNV. (C) The inhibitory effect of fluorescein isothiocyanate (FITC) -dextran (MW = 40 kDa) on corneal vessels of SD rats was observed 7 days after siRNA injection. (D) Quantification of corneal vascular area. Reproduced with permission [61]. Copyright 2016, Wiley-VCH Verlag GmbH & Co. KGaA.

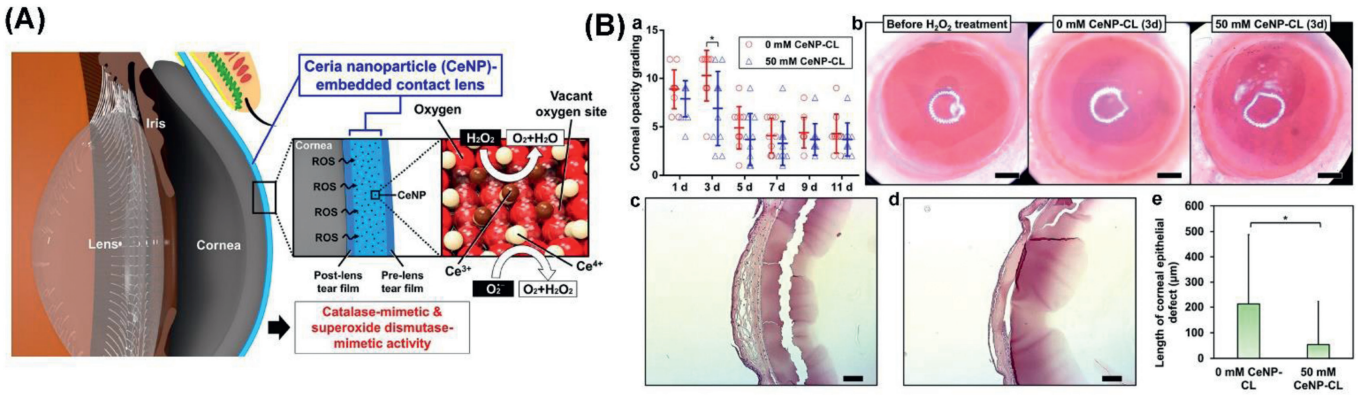


Fig. 6. (A) Diagram of ROS scavenging mechanism by CeNPs in cells [70]. Copyright 2019, The Royal Society of Chemistry. (B) Schematic diagram of CeNPs inhibiting ROS production on ocular surfaces. Reproduced with permission [71]. Copyright 2020, American Chemical Society.

CeNPs and screened their potential uses [70]. Then they confirmed that molecular composition was a significant indicator of the free radical scavenging effect of CeNPs by the inflammation-induced CNV model.

The infection of virus or bacteria can not only lead to neutrophil infiltration but also induce the production of ROS, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). In addition, neutrophil degranulation will bring further damage to corneal tissues, resulting in corneal turbidity and CNV. CeNPs can effectively reduce the production of intracellular free ROS and inflammation, thus exerting its anti-inflammatory effect and inhibiting the occurrence and development of CNV (Fig. 6A). Choi *et al.* developed ROS scavenging CeNP embedded polyhydroxyethyl methacrylate contact lens (CeNP-CLs) for the prevention of ocular surface diseases [71]. The prepared CeNP-CLs exhibit physical properties and high transparency comparable to those of commercial contact lenses, as well as excellent extracellular ROS scavenging performance (Fig. 6B). The results suggested that effective nanoparticles can be combined with existing commercial products, such as contact lenses, to move from the laboratory to the clinic and may help treat ocular surface diseases.

Moreover, Cho *et al.* detected that local administration of gold nanoparticles (AuNPs) could greatly control the formation of inflammatory CNV *via* limiting the ERK signaling pathway [72]. Mohammadpour *et al.* confirmed that the use of silica nanoparticles (SiNPs) frustrated chemical burn-induced CNV [73]. While the exact mechanism still remained unclear. Luo *et al.* synthesized gelatin-functionalized silver nanoparticles (G-Ag NPs) by simple mixture and gelatin molecules were wrapped on Ag NPs and applied in the chick chorioallantoic membrane (CAM) method (Fig. 7A) [74].

Compared with Ag NPs, G-Ag NPs showed better anti-bacterial effects due to their strong ability to destroy bacterial membrane (Fig. 7B). However, treatment with nanoparticle-impregnated cellulose discs resulted in a dramatic reduction of blood vessels (Fig. 7C). Then slit lamp observation showed that VEGF-A165 stimulation successfully initiated the centripetal growth of limbal angiogenesis. The results suggested that G-Ag NPs would be a latent

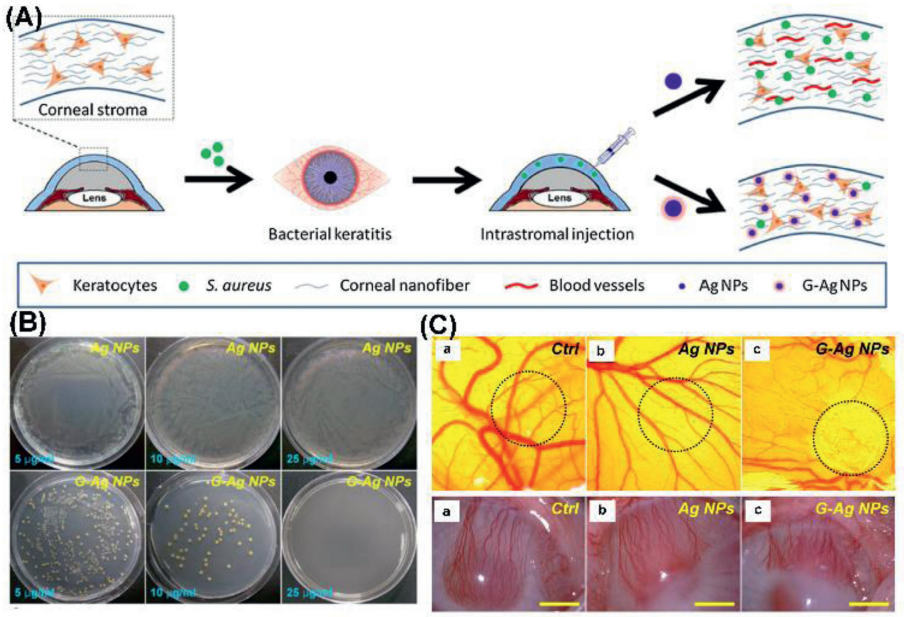


Fig. 7. (A) Schematic diagram of G-Ag NPs for treatment of bacterial keratitis. (B) Staphylococcus aureus images on LB agar plates 24 h after exposure to Ag NPs or G-Ag NPs at different concentrations. (C) After injection of PBS (a), Ag NPs (b) and G-Ag NPs (c) pretreatment cellulose filter paper for 24 h, typical light microscope images of CAM vessels and slit-lamp images of VEGF-A_{165} induced rabbit CNV 3 days later. Reproduced with permission [74]. Copyright 2018, Elsevier Inc.

bi-functional (anti-microbial and anti-angiogenic) approach for the preclinical treatment of ocular infections.

Graphene oxide (GO) nanosheets are monolayers of two-dimensional networks of sp^2 and sp^3 hybrid carbon atoms derived from graphene oxidation [75]. It has been reported that GO has a high binding affinity [76]. Considering its unique electro-optic chemical and mechanical properties, it has been applied in biotechnology for biosensors, cell imaging and anti-cancer therapy [77–80]. These applications mostly depend on their interactions with proteins. Lai *et al.* for the first time detected that bovine serum albumin- coated graphene oxide (BSA-GO) had high stability in saline and showed binding specificity to VEGF-A_{165} in composite plasma. BSA-GO could effectively frustrate the migration, proliferation and tubular formation of human umbilical vein endothelial cells (HUVEC), and block CNV induced *via* VEGF-A_{165} [81]. The results indicated that GO nanomaterials could be used as therapeutic anti-angiogenic agents *via* absorption and inhibition of VEGF activity. Thus, it may be expected to be an underlying treatment for CNV and other angiogenesis-related diseases.

5. Synergistic treatment

Photodynamic therapy (PDT) with systemic injection of verteporfin has been introduced for clinical CNV treatment, in which ROS is produced and then induces thrombotic vessel occlusions [82,83]. Photothermal therapy (PTT) can induce hyperthermic injuries to the targeted cells by converting laser radiation to local temperature increases [84]. Furthermore, the combined PDT or PTT agents can accumulate accurately in neovascularized areas, which is essential to construct efficient PDT or PTT delivery systems for CNV treatment.

Up to now, photo-therapy (PT), including PTT and PDT, has been developed as a non-invasive approach to treat a variety of diseases [85]. Similar to laser photo-coagulation, PTT or PDT could induce the damage or coagulation of blood vessels, and enable the realization of PTT or PDT-based CNV treatment [86,87]. Fossarello *et al.* investigated that two patients were treated with verteporfin-based PDT [88]. In another clinical study, You *et al.* constructed a verteporfin-based PDT with SCT of bevacizumab to treat 12 patients with CNV [89]. The results implied that successful CNV photothrombosis was achieved in both patients immediately after treatment, and neovascularization completely subsided after one repeat treatment. These findings above implied that PT could be a promising strategy in CNV treatment. However, the enrichment of small molecule drugs in CNV is low with no selectivity and limited therapeutic effects.

Based on this, our team Chu *et al.* proposed a PTT and gene-combined therapy in CNV treatment [90]. Firstly, the clinically applied indocyanine green (ICG) was assembled with dimethylpyridine amine-Zn(II) (DPA-Zn) to prepare for the DPA-Zn/ICG metal-organic nanoparticle (named nanoICG). The nanoICG showed outstanding PTT property under 808 nm laser irritation. In addition, the nanoICG modified with bis(DPA-Zn)-RGD could target newly formed blood vessels. After being loaded with survivin-siRNA, the multifunctional R-s-ICG was obtained. Meanwhile, a multimodal photoacoustic imaging (PAI) diagnostic system was constructed by PAI of oxygen saturation imaging (PA-o.s. imaging) and PAI of ICG (PAI-ICG imaging). As shown in Figs. 8A and B, R-s-ICG nanocomplexes were dropped into the eyes of CNV model rats, and an ultrasound/PAI system was used to trace the drug dynamically. By real-time multimodal PAI *in vivo* assessment of vascular changes, R-s-ICG could rapidly reach target area without residue in other normal corneal tissues. After drug administration of 24 h, the 808 nm laser irradiation was performed (Figs. 8C and D). Finally, the slit-lamp images and CD31 staining indicated that the synergistic treatment showed the best therapeutic effects. Therefore, we concluded that the synergistic treatment of PT and gene therapy (GT) could achieve an ideal treatment effect, which was significantly better than a single treatment. This provides an effective therapeutic strategy for non-invasive treatment of CNV with potential clinical transformation.

6. Conclusion and perspectives

CNV is one of the main factors of impaired vision. Therapeutic prevention of CNV is a major clinical confrontation and there is an urgent need to find enhanced and sustainable treatment [91]. Topical administration of corticosteroids remains a top priority in

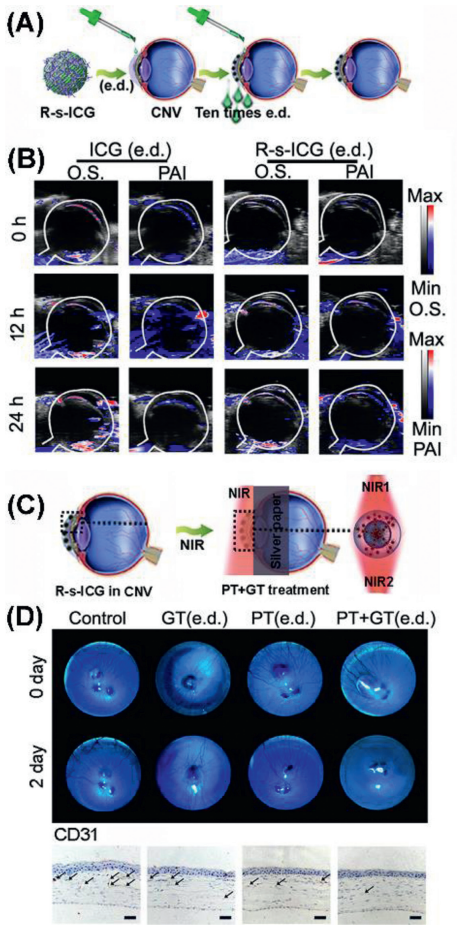


Fig. 8. (A) Schematic diagram showing R-s-ICG administration. (B) *In vivo* PA-o.s. eye imaging and PA-ICG imaging after R-s-ICG application. (C) Schematic diagram of combined photo/gene therapy for CNV. (D) Slit-lamp images and CD31 staining of CNV model eyes before and after diverse treatment. Reproduced with permission [90]. Copyright 2020, Wiley-VCH Verlag GmbH & Co. KGaA.

CNV treatment, while eyedrops may show poor bioavailability and immediate clearance [92]. With science and technology booming, pharmaceutical industry has also developed. Researchers are constantly interested in the application of nano-drug delivery systems. Moreover, further research should focus on how to design more functional, reliable and safe nanomedicine agents at the molecular level [93]. In addition, a variety of imaging methods have been used in the diagnosis of ophthalmic diseases, such as slit-lamp imaging, ICG angiography, optical coherence tomography (OCT) and fluorescein angiography. At present, molecular imaging can monitor the occurrence and development of diseases and provide timely feedback on the effects of disease treatment [94–96].

But the basic theory of nanomedicine and the preparation of nanomedicine drugs still need a lot of work, and their application in animals and in labs has not reached an advanced level. Further research is needed in the clinic to study the side effects, the safe dose, and the mechanism of action. Based on the advantages of current nanodrugs and the key scientific problems in the treatment of CNV, such as the difficulty of drug administration in the enrichment of new blood vessels and the inability to effectively monitor the treatment process, the precise clearance system of CNV designed and constructed by combining the means of medicine, pharmacy, materials science, imaging and other disciplines is an effective means to solve the problem. In the future, we hope that we can use more drug carriers to prepare ideal anti-CNV drugs through nanotechnology and multiple combinations of treatments. Also, we believe that we will find a radical drug to treat CNV.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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