Journal of Materials Science: Materials in Medicine (2025)36:43
https://doi.org/10.1007/s10856-025-06896-1

DELIVERY SYSTEMS

Original Research

Sustained intraocular pressure-lowering effect and biocompatibility of a single subconjunctival administration of hydrogel-encapsulated nano-brinzolamide

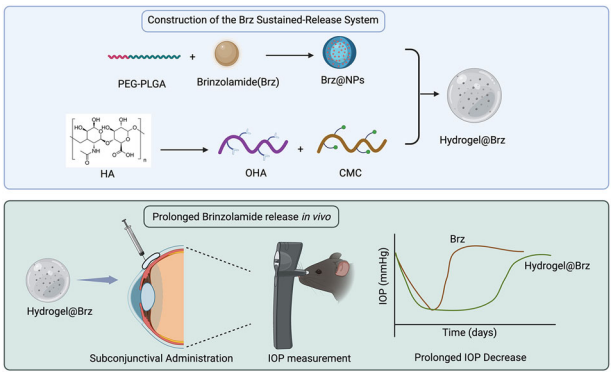
Jiahao Xu[1,2,3] · Linyu Long[4] · Xiaoyu Zhou[1] · Xinyue Zhang[1] · Li Liao[1] · Dan Ji[5] · Xuanchu Duan[1,2]

Received: 6 February 2025 / Accepted: 22 April 2025

Abstract

Brinzolamide is a widely used treatment for glaucoma, but its effectiveness relies on at least twice-daily dosing, which can be challenging for patient adherence. To overcome this limitation, we developed an injectable hydrogel-based delivery system designed to maintain therapeutic drug levels with a single administration. This approach aims to simplify treatment and improve clinical outcomes. Brinzolamide-loaded polyethylene glycol poly (lactic-co-glycolic acid) (PEG-PLGA) nanoparticles were encapsulated within a hydrogel synthesized through the crosslinking of oxidized hyaluronic acid (OHA) and carboxymethyl chitosan (CMC). In vitro studies were conducted to assess the nanoparticles' characterization, release profile, and biocompatibility. In a steroid-induced high intraocular pressure (IOP) mouse model, the efficacy of a single subconjunctival injection in lowering IOP was evaluated. Additionally, both cellular and animal biocompatibility were assessed. The brinzolamide-loaded hydrogel system (Hydrogel@Brz) contained nanoparticles with an average diameter of 40.76 nm, exhibiting a stable size distribution and a spherical morphology. The hydrogel demonstrated excellent injectability, self-healing properties, and a porous structure conducive to nanoparticle encapsulation. In vitro release studies revealed a sustained drug release of 86% over 14 days. No cytotoxicity was observed in human primary trabecular meshwork cells (HTMCs), human Tenon's capsule fibroblasts (HTFs), or the retinal ganglion cell line R28. In vivo, a single injection led to a prolonged IOP reduction lasting up to 21 days. No signs of drug toxicity were detected in ocular tissue sections, transverse optic nerve sections under transmission electron microscopy, or pathology slides of various organs. The brinzolamide-loaded hydrogel has demonstrated promising potential for sustained drug delivery and effective intraocular pressure reduction while maintaining good biocompatibility. However, further studies in larger animal models and long-term evaluations are needed to confirm its clinical applicability.

Graphical Abstract



✉ Dan Ji
475393400@qq.com

✉ Xuanchu Duan
duanxchu@csu.edu.cn

Extended author information available on the last page of the article

1 Introduction

Glaucoma is the leading cause of irreversible blindness worldwide and is characterized by progressive damage to the optic nerve. The primary strategy for managing

Published online: 20 May 2025

glaucoma is reducing intraocular pressure (IOP), which can be achieved through various treatments, including medication, laser therapy, and surgical interventions [1]. Clinicians typically prescribe eye drops from four main drug classes: beta-blockers, carbonic anhydrase inhibitors, alpha-2 adrenergic agonists, and prostaglandin analogs (PGAs). Among these, PGAs are considered an excellent first-line treatment due to their superior efficacy, safety profile, and the convenience of once-daily dosing [2]. Additionally, brinzolamide, a carbonic anhydrase inhibitor, works by reducing aqueous humor secretion and is commonly prescribed for glaucoma patients who need adjunctive therapy or cannot tolerate, or do not respond to, PGAs or beta-blockers [3, 4].

Currently, eye drop administration faces several limitations, including poor corneal penetration, rapid tear turnover, and a short drug residence time, all of which significantly reduce ocular bioavailability. To maintain therapeutic drug levels, frequent dosing is required, which can be inconvenient for patients and often leads to poor treatment adherence [5]. The development of sustained-release systems for ocular drugs is currently key to addressing this clinical issue and holds promising application prospects. At present, many prostaglandin analogs have already developed sustained-release formulations, such as Durysta [6] and iDoseTR [7], while there are fewer sustained-release systems available for brinzolamide. For example, brinzolamide-loaded core-shell nanoparticles (NPs), lipoomes and nanocapsules have been developed to enhance corneal permeability and improve drug bioavailability [8–10]; In addition, an in situ gelling ophthalmic nanoemulsion of brinzolamide, as well as brinzolamide incorporated into a niosomal in-situ gel, have been developed to achieve 24-h sustained drug release [11–13]. Most of the studies mentioned above involve drug administration via eye drops, which typically offer a short sustained-release duration. In contrast, subconjunctival drug administration can significantly prolong the release time [14]. One study in a rabbit model demonstrated that subconjunctival administration of brinzolamide encapsulated in PLGA nanoparticles provided a sustained intraocular pressure-lowering effect for up to 10 days [15]. However, a longer sustained-release duration is required for clinical application to ensure consistent therapeutic effects.

Hydrogels are extensively used in the biomedical field [16, 17]. Hydrogels, known for their strong sustained-release capabilities and high biocompatibility, have the potential to serve as effective drug carriers for reducing intraocular pressure in glaucoma treatment [18]. In this study, we encapsulated brinzolamide using PEG-PLGA nanoparticles and subsequently prepared a hydrogel cross-linked with oxidized hyaluronic acid (OHA) and carboxymethyl chitosan (CMC) to load the brinzolamide nanoparticles (Brz@NPs). This approach created a more prolonged and safer drug release system (Hydrogel@Brz). The efficacy of this system in lowering intraocular pressure and its biocompatibility were validated through material characterization, cell studies, and animal experiments. The brinzolamide-loaded hydrogel developed in this study showed promising advantages in enhancing the therapeutic efficacy of glaucoma treatment and improving patient compliance. This approach might pave the way for potential clinical translation and the development of novel therapeutic strategies for glaucoma.

2 Materials and methods

2.1 Material

Ethylene glycol, $NaIO_4$, polyvinylalcohol (PVA), nile red were obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Carboxymethyl chitosan (carboxylation degree ≥80%) and sodium hyaluronate (molecular weight: 90–100 k) were purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). The poly(-ethylene glycol) methyl ether-block-poly(lactide-co-glycolide) copolymers, featuring a 50:50 molar ratio of lactide to glycolide and designated as PEG2k-PLGA10k, were supplied by Xi'an Ruixi Biological Technology Co., Ltd (Xi'an, China).

2.2 Preparation and characterization of Brz loaded nanoparticles (Brz@NPs)

Brz-loaded PEG-PLGA nanoparticles (NPs) were prepared using an oil-in-water (o/w) emulsification–solvent evaporation method as described previously [19, 20]. In detail, 10 mg of PEG-PLGA and 3 mg of Brz were dissolved in 1 ml of dichloromethane (DCM) to form the organic phase. This organic solution was then added dropwise into 1 mL of an aqueous phase containing 1% (w/v) polyvinyl alcohol (PVA) while stirring continuously and applying sonication, thereby forming a stable o/w emulsion. The emulsion was subsequently transferred to a magnetic stirrer and maintained at room temperature for 6 h to promote complete evaporation of DCM. After the solvent had evaporated, the NP suspension was collected by centrifugation and washed 3–4 times with distilled water to remove any unencapsulated Brz and residual PVA. Finally, the purified NPs were lyophilized into a dry powder and stored at 4 °C for further analysis or use. Similarly, Nile red-loaded PEG-PLGA nanoparticles were produced using the same procedure, with Nile red substituting for Brz as the model hydrophobic fluorescent dye in the encapsulation studies.

To evaluate the encapsulation efficiency (EE) and drug loading efficiency (LE) of the NPs, a high-performance

liquid chromatography (HPLC) with UV-Vis detector was employed according to established protocols (254 nm) [21]. The NPs were suspended in deionized water at an appropriate concentration and sonicated to ensure uniform dispersion. Next, dynamic light scattering (DLS) was used to measure the particle size distribution and zeta potential, which provided insights into the size homogeneity and colloidal stability of the NPs. The morphology of the Brz-loaded NPs was characterized using transmission electron microscopy (TEM) on a Hitachi H-600 instrument (Hitachi, Japan). For TEM analysis, the NP samples were first diluted in deionized water to achieve an appropriate concentration. A droplet of the diluted suspension was then placed onto copper grids coated with a support film. The samples were negatively stained with a 2% (w/v) phosphotungstic acid solution to enhance contrast and allow clear visualization of the nanoparticle structures. After blotting away the excess stain, the grids were left to air dry. TEM imaging was performed at an acceleration voltage of 80 kV, yielding high-resolution images that detailed the size, shape, and structural characteristics of the Brz@NPs.

2.3 Preparation of oxidized hyaluronic acid (OHA)

OHA was prepared using the $NaIO_4$ oxidation method [22].One gram of hyaluronic acid (HA) was completely dissolved in 100 mL of deionized water under gentle stirring. In a separate step, 0.535 g of $NaIO_4$ was dissolved in 5 mL of distilled water, and this solution was slowly added dropwise to the HA solution while stirring continuously. The reaction mixture was then covered with aluminum foil to protect it from light and stirred at room temperature for 6 h to oxidize HA, thereby introducing aldehyde groups into its polymer chains. After the oxidation period, 1 mL of ethylene glycol was added to quench any remaining $NaIO_4$, and the mixture was stirred for an additional hour to ensure complete quenching. The resulting OHA solution was dialyzed against deionized water using a dialysis membrane with a molecular weight cut-off of 8000–14,000 Daltons. Finally, the purified OHA solution was freeze-dried to obtain a solid powder, which was stored at 4 °C for future use.

2.4 Preparation of Brz loaded OHA/CMC hydrogel

Separate 8 wt% solutions of OHA and carboxymethyl chitosan (CMC) were prepared. Brz@NPs suspensions (2 mg/mL) were then added individually to each polymer solution and stirred continuously to ensure even dispersion. Afterward, equal volumes of both solutions were combined and thoroughly mixed. The mixture was then left undisturbed until the hydrogel formation was visually observed.

2.5 Characterization of hydrogels

The surface porosity of the prepared hydrogel was characterized using scanning electron microscopy (SEM). For SEM analysis, the freeze-dried hydrogel was first rapidly frozen in liquid nitrogen for 30 min and then immediately fractured to expose its internal structure. Afterward, the sample was coated with a thin layer of gold to enhance electrical conductivity and minimize charging effects during imaging. The morphology and porous architecture were then examined using SEM.

To evaluate the shear-thinning properties of the hydrogel, shear rates ranging from 0.001 to 1000 s^{-1} were applied, and shear viscosity was measured using rotational rheometry. The hydrogel's injectability was assessed by extruding it through a 31 G syringe needle into an aqueous solution, simulating practical injection conditions and providing insight into its flow behavior under clinically relevant scenarios. Additionally, thixotropic behavior was analyzed by monitoring viscosity changes over two consecutive cycles of high (500%) and low (1%) shear strains.

In vitro drug release studies were conducted at pH 7.4 using the dialysis method [23]. Brz-loaded hydrogel was placed inside dialysis tubing and incubated in a release medium at 37 °C. At predetermined time points, aliquots were withdrawn for analysis and replaced with an equal volume of fresh medium to maintain sink conditions. The Brz concentrations in the collected samples were quantified using HPLC.

2.6 Animals

C57BL/6 male mice (8 weeks weighing about 18 g) were purchased and raised at the AIER Eye Hospital Animal Experiment Center. In order to detect the intraocular pressure-lowering effect of the drug, the animals were divided into five groups: Control group, DEX modeling group, DEX+ Hydrogel@Brz, DEX+ Brz, and DEX+ Hydrogel. All animals underwent modeling in the right eye. This study was approved by the Animal Ethics Committee of Aier Eye Hospital (AEI20230008).

2.7 Model of steroid-induced ocular hypertension and subconjunctival drug delivery intervention

Dexamethasone (DEX) (CAS 50-02-2) was purchased from APExBIO. Dexamethasone was dissolved in DMSO and saline to a final concentration of 10 mg/mL. Mice were anesthetized with 65 mg/kg of Shutai and 10 mg/kg of xylazine hydrochloride. A glass microsyringe (25 µL) was used to take 20 µL of the prepared DEX. The conjunctiva was gently pulled away from the surface of the eyeball with forceps and slowly injected for 20–25 s [24]. The high intraocular pressure

group was injected with the prepared DEX, and the control group was injected with the vehicle. Then the mice were injected once a week to maintain high intraocular pressure.

After the intraocular pressure increased, 25 μL of drug was injected subconjunctivally once (as shown in Supplementary Fig. 1). The TonoLab rebound tonometer was used to measure the intraocular pressure of mice in a non-anesthetized state. The measurement time was 12AM–2PM every day. The model was established and measured every 1–7 days according to the changes in intraocular pressure.

2.8 In vivo sustained release effect testing of materials

The sustained-release model of Nile red instead of Brz is Hydrogel@Nile red. The control group uses Nile red nanoparticles. A new batch of mice was selected and 20 μL of the drug was injected subconjunctivally at one time. The eyeballs were removed after 1 week and 2 weeks, and frozen sections were made. The fluorescence intensity was detected under Ex/Em~552/636 nm conditions.

2.9 Cytotoxicity and In vivo toxicity testing

Human Tenon's capsule fibroblasts (HTFs) (HTX2135 from Otwo Biotech Inc), Human primary trabecular meshwork cells (HTMCs) (from Aier Glaucoma Institute), and retinal ganglion cell line R28 (from Neural Repair Laboratory, The Second Affiliated Hospital of Hunan Normal University) were used to evaluate drug cytotoxicity. Sterilized Brz (1 μL, 1 μg/μL), Hydrogel (1 μL), and Hydrogel@Brz (1 μL) were directly added to 96-well plates for intervention. CCK8 cell death detection kit was used for determination after 24 h. Phalloidin was used to detect the cytoskeletal status of HTFs and HTMCs. Cytotoxicity was evaluated per ISO 10993-5 guidelines, with cell viability thresholds defined as follows: ≥70% = non-cytotoxic.

Healthy C57 mice without modeling were used and divided into a control group, a Hydrogel group, and a Hydrogel@Brz group. They were intervened for 3 weeks. On the 21nd day, visual quality and phNR wave tests were performed, and the eyeballs were removed for immunohistochemistry detection of the retinal ganglion cell marker Brn3a. The cross-sections of the optic nerves were taken for transmission electron microscopy, and the organs of the mice were taken for pathological sections to explore the toxicological status of the organs.

2.10 Data analysis

Data are expressed as mean ± standard deviation (SD). For comparisons between two groups, unpaired Student's t test or Welch's t test were applied. Statistical significance was defined as $*p < 0.05$, $**p < 0.01$, $**p < 0.001$. All analyses were performed using GraphPad Prism 9.0.

3 Results

3.1 Characterization of Brz@NPs and hydrogel

The particle size of the drug-loaded nanoparticles was 40.76 nm and the Polydispersity Index (PDI) was 0.162 (Fig. 1A) with a zeta potential of -21.33 ± 1.38 mV (Supplementary Fig. 2). Stability studies revealed that both the particle size and PDI remained consistent over a two-week period when stored at 4 °C (Fig. 1B). The transmission electron microscopy of the drug-loaded nanoparticles showed that they were quasi-circular (Fig. 1C). Subsequently, we successfully synthesized oxidized hyaluronic acid (OHA) from hyaluronic acid (HA) using sodium periodate, as confirmed by ^{1}H NMR spectroscopy (Fig. 1D). A stable hydrogel was formed within three minutes upon mixing 4 wt% OHA with 4 wt% carboxymethyl chitosan (CMC) at room temperature (Fig. 1E). Scanning electron microscopy (SEM) observations revealed that this hydrogel exhibited a porous structure (Fig. 1F).

The crosslinking mechanism based on dynamic Schiff base bonds imparted the hydrogel with excellent injectability and self-healing properties [25]. These characteristics were validated in this study using a rotational rheometer. As shear stress increased, the viscosity of the hydrogel decreased, indicating favorable shear-thinning behavior (Fig. 1G). The inset image in Fig. 1G also demonstrated that the hydrogel could be extruded from a syringe with a needle to form a stable "HG" shape. Moreover, step-strain sweep test results indicated that the hydrogel structure, which was disrupted under a strain of 500%, could rapidly recover under low-strain conditions, showcasing superior self-healing capability (Fig. 1H). The injectability of the hydrogel significantly facilitated clinical administration, while its rapid self-healing property ensured that upon subconjunctival injection, the hydrogel could quickly form a stable gel structure, thereby preventing unnecessary diffusion. In vitro drug release studies showed rapid initial release over the first two days, followed by gradual sustained release; by day 14, the cumulative release reached 86% (Fig. 1I).

In summary, we successfully developed hydrogel-loaded brinzolamide nanoparticles, referred to as Hydrogel@Brz, which exhibit good stability, excellent injectability, and a prolonged in vitro sustained release profile.

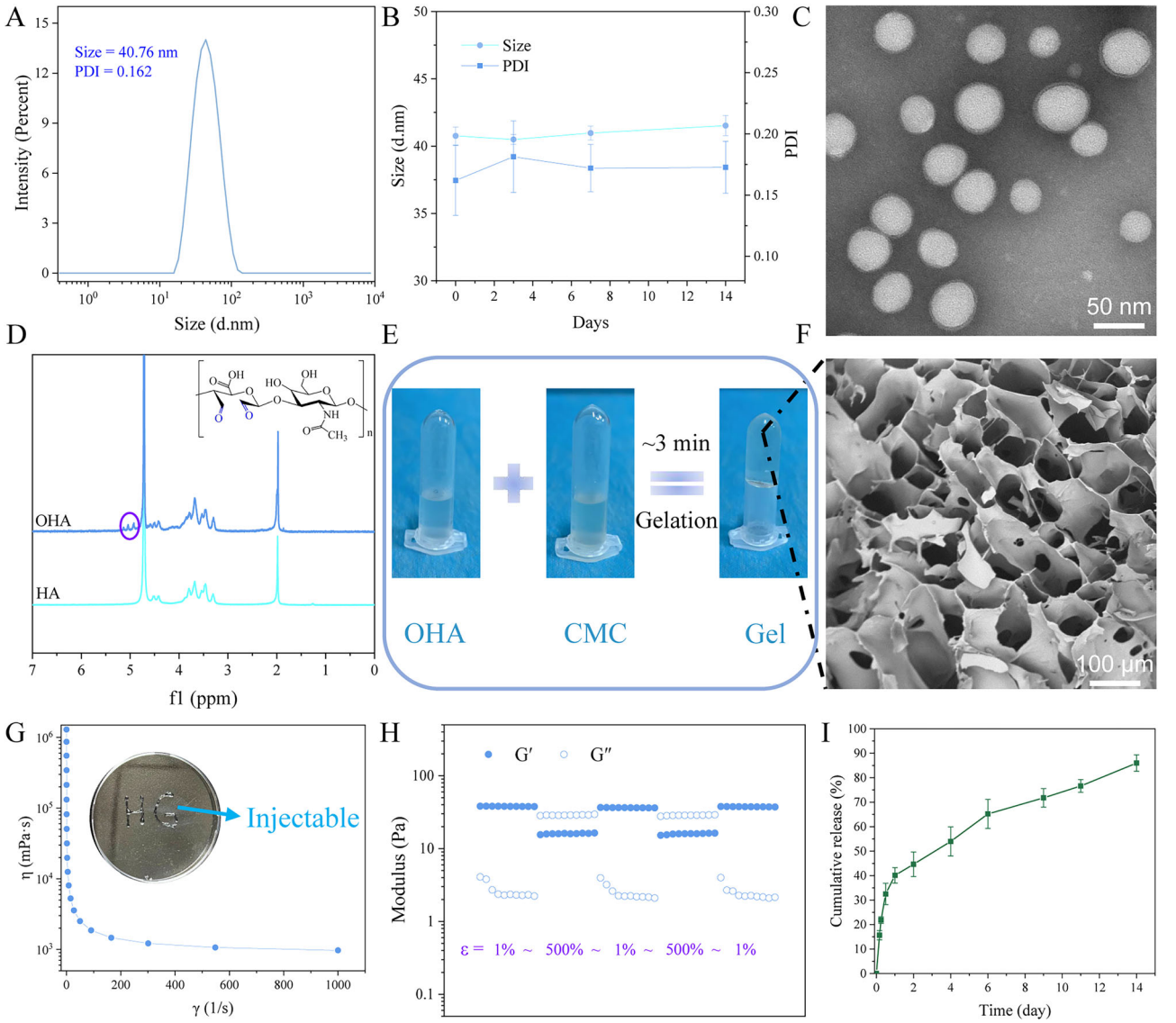


Fig. 1 Material characterization. **A** The drug-loaded nanoparticles have a particle size of 40.76 nm, a PDI of 0.162, and a uniform size distribution; **B** The particle size and PDI of the nanoparticles are stable within two weeks of storage at 4 °C; **C** Transmission electron microscopy of the nanoparticles; **D** Nuclear magnetic resonance spectrum; the three new peaks in the circle represent the successful synthesis of OHA; **E** 4 wt% OHA and 4 wt% CMC form a stable hydrogel within 3 min at room temperature; **F** Scanning electron microscopy: The hydrogel has a porous surface structure; **G** Shear thinning, the viscosity of the hydrogel decreases with the increase of shear rate, and it has good injectability; **H** Step strain scanning, when the strain is 1%, the storage modulus G′> loss modulus G″, the hydrogel structure; when the strain is 500%, the storage modulus G′< loss modulus G″, the hydrogel structure is destroyed, and when the strain is restored to 1%, the hydrogel structure can be restored. The representative hydrogel has good self-healing properties; **I** In vitro drug release, the cumulative drug release was 44.63% at two days and 86% at 14 days

3.2 The intraocular pressure-lowering effect of a single subconjunctival injection of Hydrogel@Brz

As shown in Fig. 2, the baseline intraocular pressure (IOP) of C57 mice under normal conditions was 11.64 ± 0.64 mmHg. Following DEX-induced modeling, the IOP increased significantly to around 17.65 ± 0.88 mmHg. A single subconjunctival injection led to a noticeable reduction in IOP in both the Brz group and the Hydrogel@Brz group, with the effect becoming evident 24 h after administration. In contrast, the DEX group and the Hydrogel group continued to exhibit elevated IOP levels.

By the 5th day, a sustained reduction in IOP was observed only in the Hydrogel@Brz group. On the 14th day, the average IOP in this group decreased to 13.20 ± 0.84 mmHg, significantly lower than the 18.2 ± 0.84 mmHg recorded in the DEX group. However, after 14 days, the IOP in the Hydrogel@Brz group began to gradually rise. By the 20th day, the average IOP had

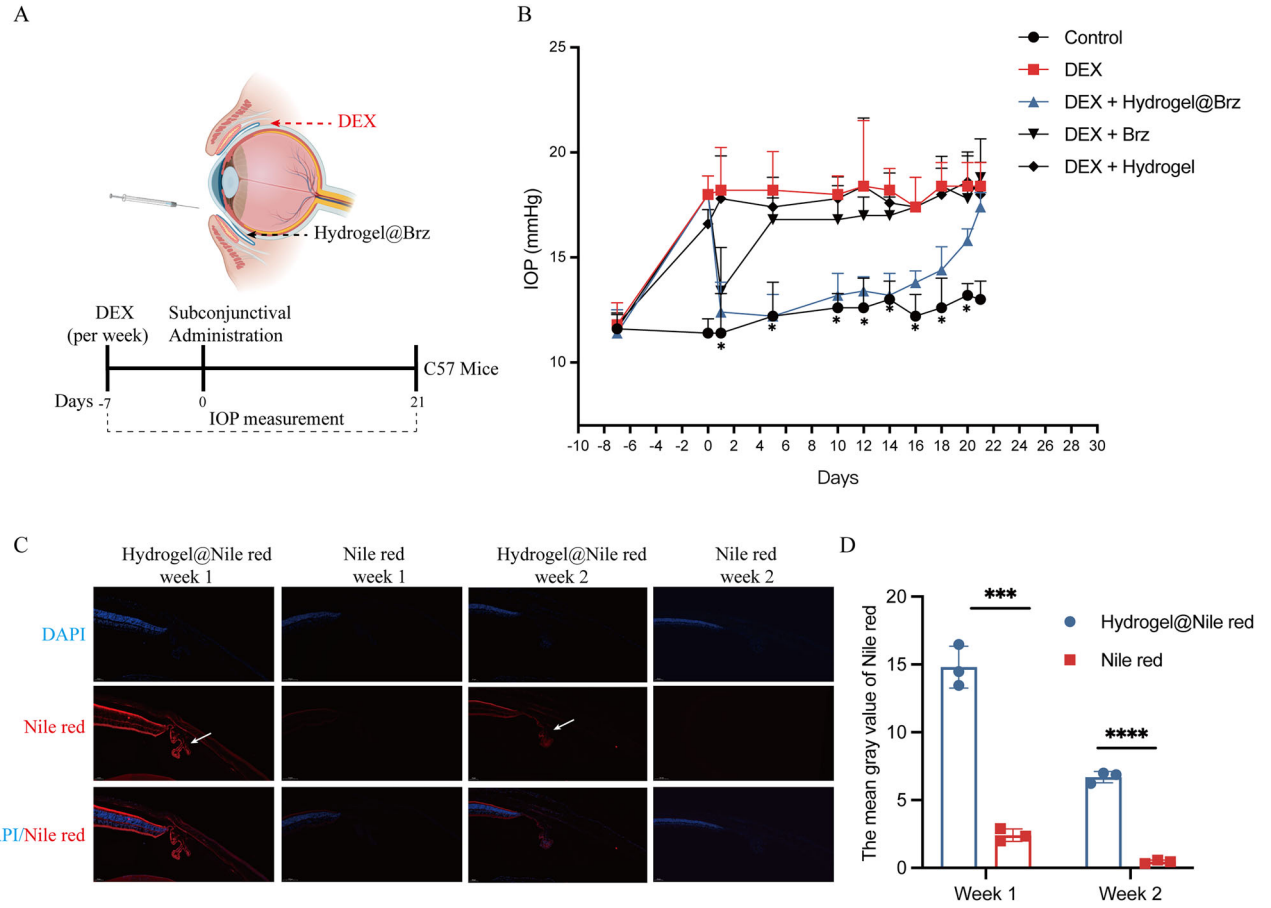


Fig. 2 Hydrogel@Brz's intraocular pressure-lowering effect and preliminary in vivo drug release investigation. **A** Schematic diagram of the modeling approach; **B** Changes in intraocular pressure; **C** Fluorescence and semi-quantitative analysis of frozen sections from eyeballs collected 1 and 2 weeks after subconjunctival injection of hydrogel-loaded Nile red nanoparticles (Week 1 Hydrogel@Nile red = 14.8 ± 1.5, Nile red = 2.4 ± 0.5; Week 2 Hydrogel@Nile red = 6.7 ± 0.4, Nile red = 0.5 ± 0.1). The white arrow points to the ciliary body that produces aqueous humor. Data analyzed by unpaired Student's *t* test or Welch's *t* test; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$

reached 15.8 ± 0.45 mmHg, still significantly lower than the 18.4 ± 0.89 mmHg observed in the DEX group. By the 21st day, although the Hydrogel@Brz group continued to show a lower IOP compared to the DEX group, the difference between the two groups was no longer statistically significant.

Brinzolamide is a lipophilic drug; however, tracking its metabolism within the eye remains challenging. In contrast, Nile red, known for its strong lipophilicity, remarkable fluorescence, and excellent imaging capabilities, serves as a valuable tool for studying the metabolic localization of drugs in vivo [26]. Therefore, we utilized hydrogel-loaded Nile red nanoparticles (Hydrogel@Nile red) to conduct a preliminary investigation of sustained drug release in vivo. In a separate batch of unmodeled C57 mice, Hydrogel@Nile red and a Nile red control solution were injected subconjunctivally. Eyeballs were collected at 7 and 14 days for frozen sectioning and fluorescence imaging to observe the distribution and metabolism of Nile red.

Fluorescence imaging revealed that Nile red was distributed in the ciliary body. At day 7, the mean gray value in the Hydrogel@Nile red group was significantly higher than that in the Nile red control group. By day 14, Nile red in the control group was almost completely metabolized, whereas a substantial amount remained in the Hydrogel@Nile red group (Fig. 2C, D). These findings suggest that the sustained-release system provides drug release for at least 14 days in vivo, which may correspond to the 21-day intraocular pressure-lowering effect observed in this study.

3.3 Drug cytotoxicity assay

Cytotoxicity experiments demonstrated that, compared to the control group, the relative cell viability in all drug-treated groups remained above 70%. This indicates that specific concentrations of Brz, Hydrogel, and Hydrogel@Brz did not induce significant cytotoxic effects on

Human Tenon's capsule fibroblasts (HTFs), human primary trabecular meshwork cells (HTMCs), or the retinal ganglion cell line R28 (Fig. 3A–C). Additionally, no noticeable alterations in the cytoskeleton were observed in the Brz, Hydrogel, and Hydrogel@Brz groups compared to the control group, indicating that the drug maintains a certain level of cellular safety (Fig. 3D, E).

3.4 Animal toxicology experiments

Animal toxicology studies were conducted on a cohort of healthy C57 mice, which were divided into three groups: the control group, the Hydrogel group, and the Hydrogel@Brz group (Fig. 4A). After 21 days of intervention, visual quality assessments showed no significant differences among the three groups (Fig. 4B). Similarly, phNR wave

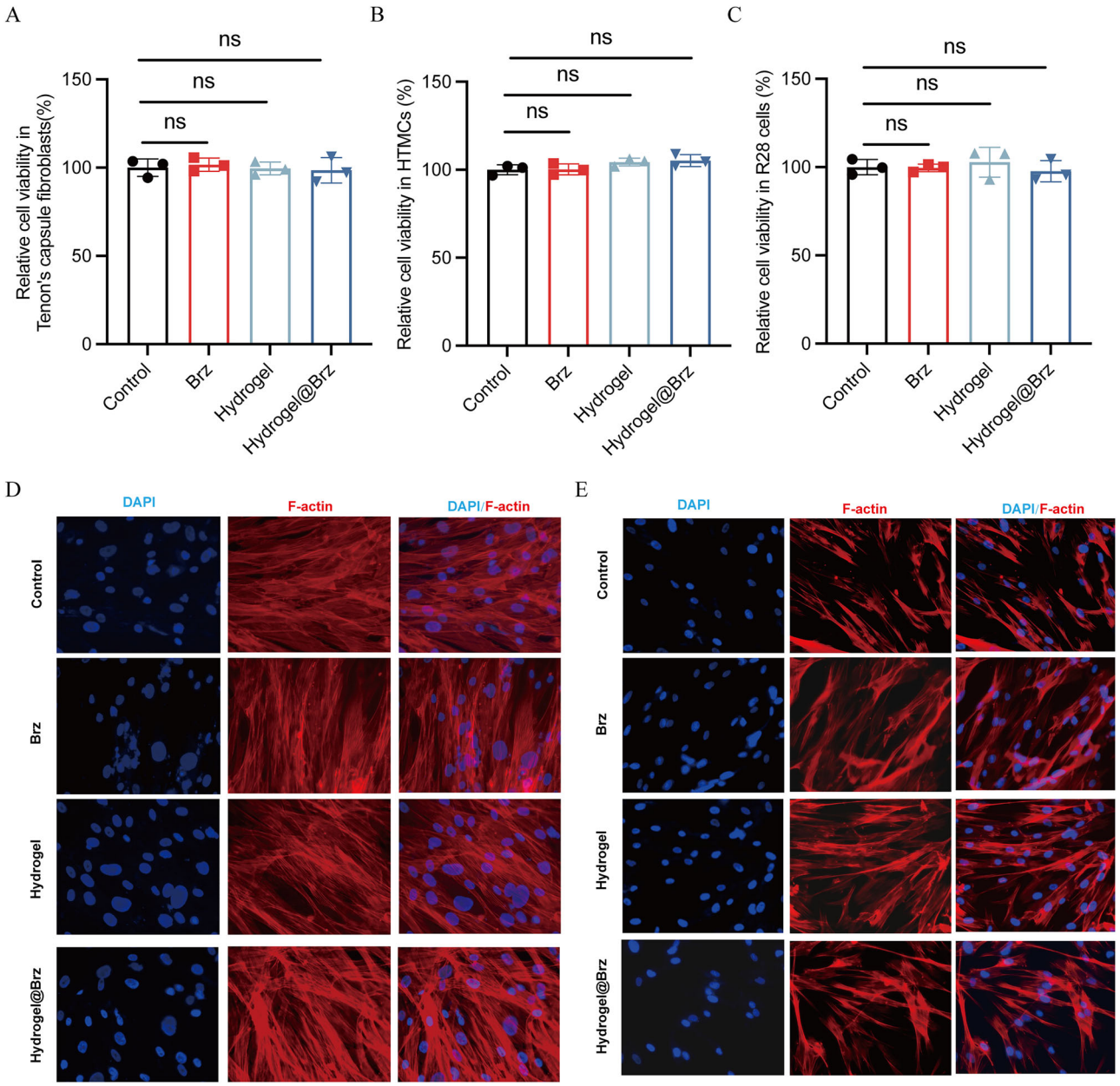


Fig. 3 Cytotoxicity of the drug. **A–C** CCK8 assay evaluating the relative cell viability of HTFs (Control = 100.0 ± 5.0, Brz = 101.7 ± 3.8, Hydrogel = 99.6 ± 3.6, Hydrogel@Brz = 98.5 ± 7.1), HTMCs (Control = 100.0 ± 2.8, Brz = 100.2 ± 3.1, Hydrogel = 104.3 ± 2.2, Hydrogel@Brz = 105.2 ± 3.34), and R28 cells (Control = 100.0 ± 4.3, Brz = 99.54 ± 2.1, Hydrogel = 102.8 ± 8.4, Hydrogel@Brz = 97.7 ± 6.0); **D**, **E** Phalloidin staining showing cytoskeletal structures of HTMCs and HTFs after drug intervention. Data analyzed by unpaired Student's *t* test or Welch's *t* test; ns = $p > 0.05$

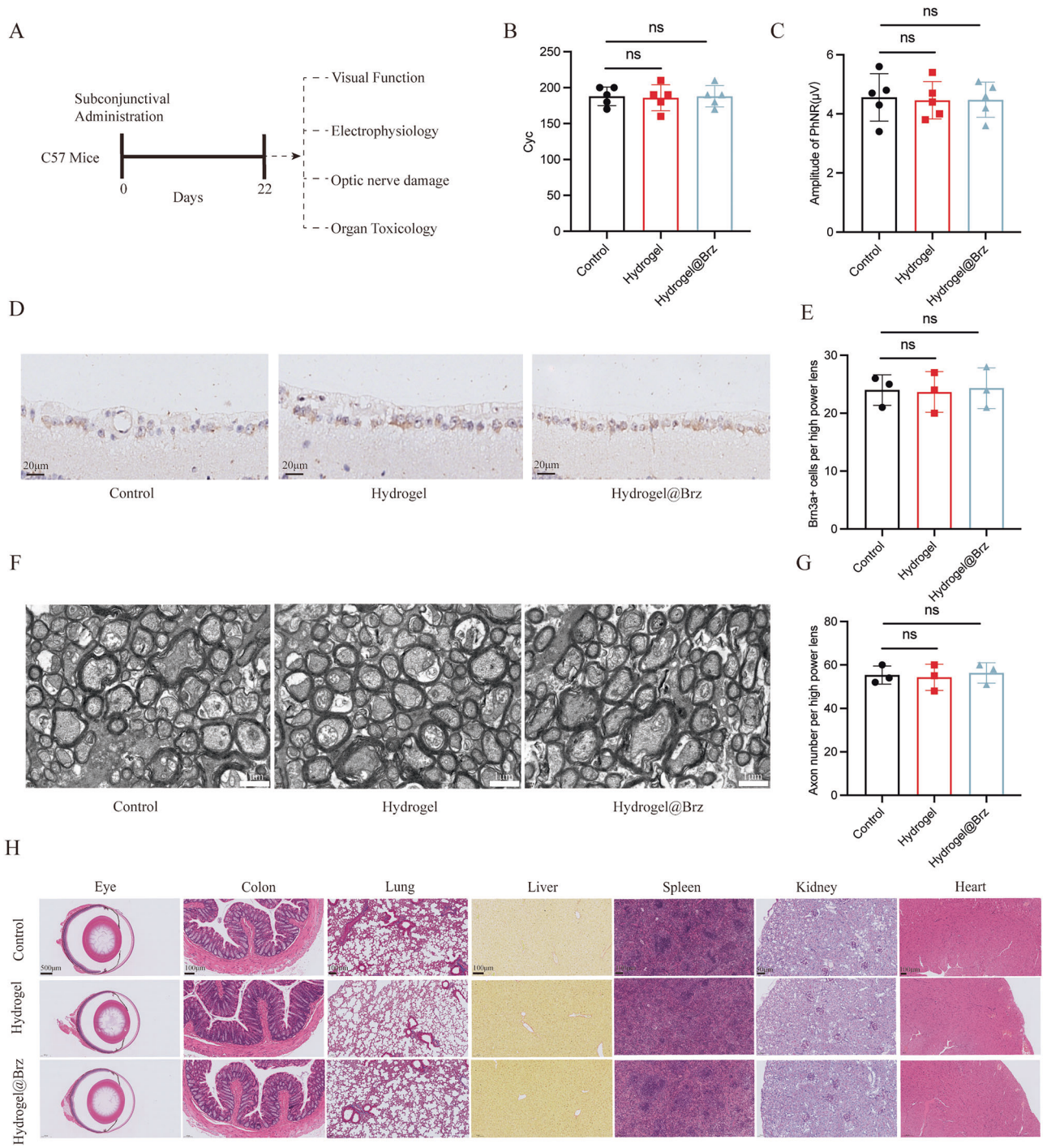


Fig. 4 Biocompatibility at the animal level. **A** Schematic diagram of animal toxicology experiments; **B** Visual performance analysis, where Cyc(Cycles) represents mouse visual ability, with higher values indicating better performance (Control = 188.0 ± 13.0, Hydrogel = 186.0 ± 18.2, Hydrogel@Brz = 188.0 ± 14.8); **C** Electrophysiological assessment of phNR wave amplitude, which decreases in cases of retinal ganglion cell damage (Control = 4.56 ± 0.80, Hydrogel = 4.46 ± 0.63, Hydrogel@Brz = 4.48 ± 0.60); **D**, **E** Immunohistochemical staining and semi-quantitative analysis of the retinal ganglion cell marker Brn3a (Control = 24.0 ± 2.7, Hydrogel = 23.67 ± 3.5, Hydrogel@Brz = 24.33 ± 3.5); **F**, **G** Transmission electron microscopy of optic nerve cross-sections and axon counting (Control = 55.3 ± 4.2, Hydrogel = 54.3 ± 6.0, Hydrogel@Brz = 56.3 ± 4.7); H. Histological sections and staining of the eyeball and major systemic organs. Staining includes HE for the eyeball, intestine, spleen, and heart; Masson's trichrome for the lungs; Sirius Red for the liver; and PAS staining for glycogen in the kidneys. Data analyzed by unpaired Student's *t* test or Welch's *t* test; ns = $p > 0.05$

measurements revealed no notable variations (Fig. 4C). To further assess potential toxicity, the eyeballs and optic nerves of the mice were collected for analysis. Immunohistochemical staining showed no significant differences in Brn3a expression within the retinal ganglion cell layer across groups (Fig. 4D, E). Additionally, transmission electron microscopy of optic nerve cross-sections indicated no significant changes in axon density (Fig. 4F, G). Further pathological analysis was conducted on key organs, including the eyes, heart, liver, spleen, lungs, kidneys, and intestines (Fig. 4H). Histological examination revealed no structural abnormalities or immune cell infiltration in any of these tissues. Moreover, no adverse effects were observed in key liver and kidney function indicators (Supplementary Fig. 3).

4 Discussion

Glaucoma remains a leading cause of irreversible blindness globally, with intraocular pressure (IOP) reduction serving as the cornerstone of treatment. While prostaglandin analogs (PGAs) are widely recognized as first-line therapies due to their efficacy and once-daily dosing [27], adjunctive agents such as brinzolamide—a carbonic anhydrase inhibitor—are essential for patients intolerant to PGAs or requiring additional IOP control [28]. Despite their therapeutic value, conventional eye drops face limitations including poor bioavailability, short residence time, and patient non-adherence [29]. To address these challenges, sustained-release drug delivery systems have emerged as a transformative approach. This study introduces a novel hydrogel-based sustained-release system (Hydrogel@Brz), which combines brinzolamide-loaded PEG-PLGA nanoparticles (Brz@NPs) with an oxidized hyaluronic acid (OHA)/carboxymethyl chitosan (CMC) hydrogel. The system exhibited prolonged IOP reduction, excellent injectability, and good biocompatibility, making it a potential alternative to traditional glaucoma therapies.

The Hydrogel@Brz system achieved sustained drug release over 14 days in vitro and maintained IOP reduction for up to 21 days in vivo, a significant improvement over existing brinzolamide formulations. The prolonged efficacy observed in this study is likely attributed to the dual mechanisms of PEG-PLGA nanoparticles and the hydrogel matrix, which work together to enhance sustained drug release and retention. PEG-PLGA nanoparticles, with their small size and negative zeta potential, construct a stable drug loading structure. Meanwhile, the OHA/CMC hydrogel forms a porous network, enabling gradual nanoparticle release. The shear-thinning and self-healing properties of the hydrogel further ensure clinical practicality, allowing smooth injection through a needle and rapid structural recovery post-administration. These features align with recent advances in injectable hydrogels for ocular drug delivery [30], yet our system uniquely integrates nanoparticle encapsulation with a tunable hydrogel to extend release duration.

The in vivo Nile red tracking experiments corroborated the sustained-release profile, showing fluorescence retention in the ciliary body for 14 days in the Hydrogel@Nile red group, whereas the control group displayed rapid clearance. This localization is crucial, as brinzolamide exerts its IOP-lowering effect by inhibiting carbonic anhydrase in the ciliary body, where it plays a key role in aqueous humor production [31]. The correlation between sustained drug presence and prolonged IOP reduction suggests that Hydrogel@Brz effectively maintains therapeutic concentrations at the target site. However, the gradual IOP rebound after day 14 highlights the need for hydrogel formulation optimization, but balancing prolonged release with injectability remains a challenge [32], increasing crosslinking density could extend release but may compromise clinical applicability. Future studies should explore structural modifications, such as incorporating additional polymer layers or adjusting the OHA/CMC ratio, to further prolong efficacy without sacrificing functionality.

A critical advantage of Hydrogel@Brz lies in its biocompatibility. Cytotoxicity assays revealed no adverse effects on human Tenon's fibroblasts (HTFs), trabecular meshwork cells (HTMCs), or retinal ganglion cells (R28). This is particularly significant for HTFs, as subconjunctival fibrosis remains a major cause of failure in glaucoma surgeries [33]. The absence of cytoskeletal disruption or fibrotic changes in HTFs suggests that the hydrogel may mitigate postoperative scarring, a hypothesis warranting further investigation. The absence of cytotoxicity aligns with ISO 10993-5 guidelines and is consistent with findings in chitosan-PEG hybrid hydrogels [34]. Similarly, in vivo toxicology studies demonstrated preserved retinal function and structure despite prolonged hydrogel exposure, suggesting minimal off-target effects—a key characteristic of advanced ocular drug delivery systems [35]. While CMC and OHA in the hydrogel are known for their low immunogenicity [36], the potential for hypersensitivity reactions must be carefully considered in clinical translation.

While this study demonstrates the feasibility of Hydrogel@Brz, several limitations must be addressed. First, the murine model's small ocular volume precluded direct measurement of aqueous humor brinzolamide concentrations. Larger animal models, such as rabbits—which share closer anatomical and physiological similarities with humans—are necessary to quantify pharmacokinetic parameters [37], Future studies in larger animal models will incorporate standardized clinical scoring (e.g., Draize test), behavioral monitoring to complement histopathological

findings and the correlation between hydrogel degradation and brinzolamide release profile to improve treatment efficacy, further promoting clinical translation; Second, while the current formulation's 21-day efficacy surpasses existing options, it may still fall short of the ideal 3 to 6-month duration required for true clinical convenience. Optimizing hydrogel degradation rates through innovations such as enzyme-responsive crosslinkers or pH-sensitive modifications [38, 39], could further extend drug release and enhance its clinical applicability; Third, while subconjunctival administration minimizes systemic exposure, the 21-day efficacy period only meets the criteria for subacute systemic toxicity in rodents, future studies should extend observation to 6 months to comply with chronic toxicity standards. Such evaluations will further validate the hydrogel's safety for potential human trials.

5 Conclusion

Glaucoma intraocular pressure-lowering therapy remains the first-line treatment for glaucoma, and the development of sustained-release drug delivery systems holds significant clinical value. However, research on sustained-release formulations for the commonly used IOP-lowering drug brinzolamide remains limited. In this study, we developed a hydrogel-loaded brinzolamide nanoparticle system that, with a single subconjunctival injection, achieved a sustained IOP-lowering effect for up to 21 days in a mouse model. Furthermore, its basic biocompatibility was confirmed at both the cellular and animal levels. This research suggests potential for clinical translation and offers insights for the future development of brinzolamide sustained-release systems.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10856-025-06896-1.

Acknowledgements We thank all the mice that were dedicated to this experiment. The graphical abstract figure was created in BioRender. Xu, J. (2024) https://BioRender.com/x61e765.

Author contributions Jiahao Xu and Xuanchu Duan designed the research; Jiahao Xu and Dan Ji wrote and revised the manuscript; Linyu Long provided the materials and technical support; Xiaoyu Zhou, Xinyue Zhang and Li Liao participated the animal experiments.

Funding This work was supported by National Natural Science Foundation of China (grant numbers 82401262 to Xiaoyu Zhou, 82471079 to Xuanchu Duan); Hunan Engineering Research Center for Glaucoma with Artificial Intelligence in Diagnosis and Application of New Materials [grant numbers 2023TP2225 to Xuanchu Duan]; the Natural Science Foundation of Hunan Province, China [grant numbers 2023JJ40004 to Xiaoyu Zhou, 2023JJ70014 to Xuanchu Duan]; the Science and Technology Foundation of Aier Eye Hospital Group, China [grant numbers AMF2406D02 to Jiahao Xu]; the Natural Science Foundation of Changsha [grant numbers kq2403205 to Jiahao Xu].

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

List of abbreviations

Brz	brinzolamide
DEX	Dexamethasone
POAG	Primary open angle glaucoma
IOP	Intraocular pressure
HA	hyaluronic acid
OHA	oxidized hyaluronic acid
CMC	carboxymethyl chitosan
PEG	Polyethylene Glycol
PLGA	Poly (lactic-co-glycolic acid)
HTMCs	Human primary trabecular meshwork cells
HTFs	Human Tenon's capsule fibroblasts
SEM	Scanning electron microscopy
HPLC	High-Performance Liquid Chromatography

References

1. Jonas JB, Aung T, Bourne RR, Bron AM, Ritch R, Panda-Jonas S. Glaucoma. Lancet. 2017;390:2183–93.
2. Holló G, Katsanos A, Boboridis K, Irkec M, Konstas A. G. P. Preservative-free prostaglandin analogs and prostaglandin/timolol fixed combinations in the treatment of glaucoma: efficacy, safety and potential advantages. Drugs. 2018;78:39–64.
3. Lusthaus JA, Goldberg I. Brimonidine and brinzolamide for treating glaucoma and ocular hypertension; a safety evaluation. Expert Opinion Drug Safety. 2017;16:1071–8.
4. Li T, Lindsley K, Rouse B, Hong H, Shi Q, Friedman DS, et al. Comparative effectiveness of first-line medications for primary open-angle glaucoma: a systematic review and network meta-analysis. Ophthalmology. 2016;123:129–40.
5. Cholkar K, Trinh HM, Pal D, Mitra AK. Discovery of novel inhibitors for the treatment of glaucoma. Expert Opin Drug Discov. 2015;10:293–313.
6. Shirley M. Bimatoprost implant: first approval. Drugs Aging. 2020;37:457–462.

7. iDose TR - a travoprost implant for glaucoma. Med Lett Drugs Ther. 2024;66:70-71.
8. Song J, Zhang Z. Brinzolamide loaded core-shell nanoparticles for enhanced coronial penetration in the treatment of glaucoma. J Appl Biomater Funct Mater. 2020;18:2280800020942712.
9. Dubey V, Mohan P, Dangi JS, Kesavan K. Brinzolamide loaded chitosan-pectin mucoadhesive nanocapsules for management of glaucoma: Formulation, characterization and pharmacodynamic study. Int J Biological Macromolecules. 2020;152:1224–32.
10. Li H, Liu Y, Zhang Y, Fang D, Xu B, Zhang L, et al. Liposomes as a novel ocular delivery system for brinzolamide: in vitro and in vivo studies. AAPS PharmSciTech. 2016;17:710–7.
11. Bhalerao H, Koteshwara KB, Chandran S. Design, optimisation and evaluation of in situ gelling nanoemulsion formulations of brinzolamide. Drug Deliv Transl Res. 2020;10:529–47.
12. Bhalerao H, Koteshwara KB, Chandran S. Brinzolamide dimethyl sulfoxide in situ gelling ophthalmic solution: formulation optimisation and in vitro and in vivo evaluation. AAPS PharmSciTech. 2020;21:69.
13. Gupta P, Yadav KS. Formulation and evaluation of brinzolamide encapsulated niosomal in-situ gel for sustained reduction of IOP in rabbits. J Drug Deliv Sci Technol. 2022;67:103004.
14. Li J, Tian Q, Sun H, Zhang Y, Yang X, Kaur P, et al. A novel, liposome-loaded, injectable hydrogel for enhanced treatment of choroidal neovascularization by sub-tenon's injection. Mater Today Nano. 2022;20:100264.
15. Subrizi A, Del Amo EM, Korzhikov-Vlakh V, Tennikova T, Ruponen M, Urtti A. Design principles of ocular drug delivery systems: importance of drug payload, release rate, and material properties. Drug Discov Today. 2019;24:1446–57.
16. Li Y, Chen Y, Xue Y, Jin J, Xu Y, Zeng W, et al. Injectable hydrogel delivery system with high drug loading for prolonging local anesthesia. Adv Sci. 2024;11:e2309482.
17. Hu B, Gao M, Boakye-Yiadom KO, Ho W, Yu W, Xu X, et al. An intrinsically bioactive hydrogel with on-demand drug release behaviors for diabetic wound healing. Bioact Mater. 2021;6:4592–606.
18. Patel KD, Barrios Silva L, Park Y, Shakouri T, Keskin-Erdogan Z, Sawadkar P, et al. Recent advances in drug delivery systems for glaucoma treatment. Materials Today Nano. 2022;18:100178.
19. Zhou Y, Fang A, Wang F, Li H, Jin Q, Huang L, et al. Core-shell lipid-polymer nanoparticles as a promising ocular drug delivery system to treat glaucoma. Chin Chem Lett. 2020;31:494–500.
20. Zhao P, Wang H, Yu M, Liao Z, Wang X, Zhang F, et al. Paclitaxel loaded folic acid targeted nanoparticles of mixed lipid-shell and polymer-core: in vitro and in vivo evaluation. Eur J Pharm Biopharm. 2012;81:248–56.
21. Wu W, Li J, Wu L, Wang B, Wang Z, Xu Q, et al. Ophthalmic delivery of brinzolamide by liquid crystalline nanoparticles: in vitro and in vivo evaluation. AAPS PharmSciTech. 2013;14:1063–71.
22. Park DJ, Kim SC, Jang JB, Lee B, Lee S, Ryu B, et al. Multifunctional hydrogel dressing based on fish gelatin/oxidized hyaluronate for promoting diabetic wound healing. J Mater Chem B. 2024;12:4451–66.
23. Sadeghi-Abandansari H, Pakian S, Nabid M-R, Ebrahimi M, Rezalotfi A. Local co-delivery of 5-fluorouracil and curcumin using Schiff's base cross-linked injectable hydrogels for colorectal cancer combination therapy. Eur Polym J. 2021;157:110646.
24. Patel GC, Phan TN, Maddineni P, Kasetti RB, Millar JC, Clark AF, et al. Dexamethasone-induced ocular hypertension in mice: effects of myocilin and route of administration. Am J Pathol. 2017;187:713–23.
25. Chen J, Zhu H, Zhu Y, Zhao C, Wang S, Zheng Y, et al. Injectable self-healing hydrogel with siRNA delivery property for sustained STING silencing and enhanced therapy of intervertebral disc degeneration. Bioact Mater. 2021;9:29–43.
26. Valverde C, Lligadas G, Ronda JC, Galià M, Cádiz V. Synthesis and characterization of castor oil-derived oxidation-responsive amphiphilic block copolymers: Poly(ethylene glycol)-b-poly(11-((2-hydroxyethyl)thio)undecanoate). Eur Poly J. 2020;133:109736.
27. Zhou L, Zhan W, Wei X. Clinical pharmacology and pharmacogenetics of prostaglandin analogues in glaucoma. Front Pharmacol. 2022;13:1015338.
28. Liu Y, Zhao J, Zhong X, Wei Q, Huang Y. Efficacy and Safety of brinzolamide as add-on to prostaglandin analogues or β-blocker for glaucoma and ocular hypertension: a systematic review and meta-analysis. Front Pharmacol. 2019;10:679.
29. Moore SG, Richter G, Modjtahedi BS. Factors affecting glaucoma medication adherence and interventions to improve adherence: a narrative review. Ophthalmol Ther. 2023;12:2863–80.
30. Akulo KA, Adali T, Moyo MTG, Bodamyali T. Intravitreal injectable hydrogels for sustained drug delivery in glaucoma treatment and therapy. Polymers. 2022;14:2359.
31. Jiang J, Kong K, Fang X, Wang D, Zhang Y, Wang P, et al. CRISPR-Cas9-mediated deletion of carbonic anhydrase 2 in the ciliary body to treat glaucoma. Cell Rep Med. 2024;5:101524.
32. Ilochonwu BC, Urtti A, Hennink WE, Vermonden T. Intravitreal hydrogels for sustained release of therapeutic proteins. J Control Rel. 2020;326:419–41.
33. Wang Y, Geng X, Sun X, Cui H, Guo Z, Chu D, et al. Celastrol alleviates subconjunctival fibrosis induced by silicone implants mimicking glaucoma surgery. Eur J Pharm Biopharm. 2024;201:114352.
34. Jung HY, Le Thi P, Hwangbo KH, Bae JW, Park KD. Tunable and high tissue adhesive properties of injectable chitosan based hydrogels through polymer architecture modulation. Carbohydr Polym. 2021;261:117810.
35. Gabai A, Zeppieri M, Finocchio L, Salati C. Innovative strategies for drug delivery to the ocular posterior segment. Pharmaceutics. 2023;15:1862.
36. Geng X, Wang Y, Cui H, Li C, Cheng B, Cui B, et al. Carboxymethyl chitosan regulates macrophages polarization to inhibit early subconjunctival inflammation in conjunctival injury. Int J Biol Macromol. 2023;244:125159.
37. Naageshwaran V, Bigonne H, Gum G, Malla S, Del Sol C, Bon C, et al. Topical pharmacokinetics of brinzolamide suspensions in rabbits and variability analysis for sample size and design considerations. Int J Pharm. 2023;642:123183.
38. Liu HY, Korc M, Lin CC. Biomimetic and enzyme-responsive dynamic hydrogels for studying cell-matrix interactions in pancreatic ductal adenocarcinoma. Biomaterials. 2018;160:24–36.
39. Jalalvandi E, Shavandi A. In situ-forming and pH-responsive hydrogel based on chitosan for vaginal delivery of therapeutic agents. J Mater Sci Mater Med. 2018;29:158.

Affiliations

Jiahao Xu[1,2,3] · Linyu Long[4] · Xiaoyu Zhou[1] · Xinyue Zhang[1] · Li Liao[1] · Dan Ji[5] · Xuanchu Duan[1,2]

[1] Aier Glaucoma Institute, Hunan Engineering Research Center for Glaucoma with Artificial Intelligence in Diagnosis and Application of New Materials, Changsha Aier Eye Hospital, Changsha City 410015 Hunan, China

[2] Aier Academy of Ophthalmology, Central South University, Changsha, China

[3] The Xiangya Hospital, Central South University, Changsha City, Hunan, China

[4] Aier Eye Hospital Group Co. Ltd., Changsha, China

[5] Department of Ophthalmology, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha, China