• Basic Research •

N-acylethanolamine acid amidase inhibitor-loaded polymeric micelles in the treatment of dry eye disease

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Abstract

● **AIM:** To investigate F96, a N-acylethanolamine acid amidase (NAAA) inhibitor, as a novel drug for treating dry eye disease (DED) and to enhance its corneal retention time by utilizing nanometer micelles to improve therapeutic efficacy.

● **METHODS:** The study compared nanomicelles encapsulating doxorubicin with an aqueous solution of doxorubicin to assess the ability of the nanomicelles to prolong drug retention on the ocular surface. Dry eye was induced in mice through subcutaneous injections of scopolamine hydrobromide. The efficacy of F96 was evaluated using various clinical assessments, including the phenol red cotton test, Oregon green dextran staining, periodic acid-Schiff (PAS) staining, and Terminal dUTP Nick-End Labeling (TUNEL) assay.

● **RESULTS:** Doxorubicin micelles exhibited significantly prolonged retention compared to the aqueous solution. By 15min, the corneal fluorescence intensity of the micelle group was markedly higher than that of theaqueous solution group (P<0.05), and this enhanced effect persisted for at least 4h. Furthermore, mice treated with F96 demonstrated superior outcomes in tear production, corneal staining, and goblet cell density compared to the control groups. Specifically, F96-mPPP significantly increased tear secretion (3.35±0.45 *vs* 1.85±0.51 mm in the vehicle group, P<0.001), restored conjunctival goblet cell density (54.5±4.5 *vs* 31.3±3.0, P<0.01), and reduced corneal fluorescein staining scores (3.4±0.32 *vs* 6.5±0.72, P<0.001). Additionally, F96-mPPP treatment markedly decreased TUNEL-positive cells in the corneal epithelium, indicating suppression of apoptosis.

● **CONCLUSION:** F96 nanometer micelles have the potential to serve as a promising novel approach for effectively alleviating ocular surface damage in the treatment of dry eye disease.

● **KEYWORDS:** dry eye disease; F96; retention time; micelles

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INTRODUCTION

Dry eye disease (DED) is a chronic and progressive ocular disorder characterized by the disruption of tear film homeostasis, inflammation, chronic pain, and visual disturbances[1-2]. The increasing prevalence of DED affects approximately 50% of the global adult population[3-4], making it a serious public health concern. New approaches in DED therapy focused on regulating the ocular surface environment from multi-targeted treatment, including tear secretion promotion and inflammation management[5].

Palmitoylethanolamide (PEA) is a well-known anti-inflammatory and pro-resolution lipid mediator. Previous reports have highlighted the beneficial effects of PEA in various ocular diseases, including glaucoma[6], retinal neovascularization[7], diabetic retinopathy[8], and DED[9]. Supplementation with exogenous PEA has been shown to inhibit ocular surface inflammation and significantly increase

tear secretion[9]. It is important to note that enhanced PEA is rapidly metabolized to palmitic acid (PA) and ethanolamine by N-acylethanolamine acid amidase (NAAA) to terminate its signaling activity[10]. Moreover, excessive accumulation of PA on the ocular surface may exacerbate inflammation by downregulating peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α expression[11]. Inhibiting NAAA has been proposed as a strategy to alleviate inflammation by preserving the protective effects of PEA while reducing PA production[11]. NAAA is a cysteine enzyme that predominantly expressed in immune cells such as macrophages, mast cells, and lymphocytes[12]. It has been identified as a potential target for treating inflammation and related conditions. Various NAAA inhibitors have been developed and demonstrated to have anti-inflammatory and analgesic properties in animal models[13-14]. However, the use of NAAA inhibitors as a therapeutic approach for DED has not been explored extensively. A significant challenge lies in the hydrophobic properties of NAAA inhibitors, which complicates their delivery as topical eye drops.

Due to the short retention time on the ocular surface, rapid clearance from the precorneal region, and minimal corneal permeability, the ocular bioavailability of conventional anti-DED formulations is typically below 5% following topical administration[15]. The corneal barrier presents a significant challenge for most ophthalmic drug delivery[16]. Addressing the issues of poor water solubility and permeability is crucial. Polymer micelles have emerged as a promising solution for enhancing drug delivery in the treatment of eye diseases, owing to the presence of physiological barriers and elimination mechanisms in the eyes[17]. For example, a water-soluble cyclosporin A (CyA) mixed micelles formulation was developed using Pluronic® F127 as excipients and D-α tocopherol acid polyethylene glycol 1000 succinate. This formulation significantly increased the solubility and corneal concentration of highly lipophilic CyA compared to the commercially available ophthalmic emulsion Ikervis®[18]. Preclinical studies, both *in vitro* and *in vivo*, have demonstrated the favorable performance of polymeric micelles, characterized by their small size, increased tissue permeability, improved water solubility, and enhanced drug stability. Thus, polymeric micelles have been proposed as a novel strategy for ocular drug delivery to enhance the bioavailability of lipophilic agents.

This study aimed to investigate the preparation of polymeric micelles encapsulating F96, and evaluate their effectiveness for the treatment of DED. An amphiphilic multiblock copolymer, poly(ether ester urethane)s composed of poly(ethylene glycol), poly(propylene glycol), and poly(ε-caprolactone) (m-PEG-PPG-PCL), was chosen as the drug carrier. The experimental results revealed that NAAA inhibition by micelles showed a promising therapeutic approach for DED treatment.

MATERIALS AND METHODS

Ethical Approval All animal experiments were approved by the Laboratory Animal Center at Xiamen University (XMULAC20230055). The treatment of all mice was in accordance with the guidelines set forth by Association of Research and Vision in Ophthalmology (ARVO).

Materials F96 is a novel NAAA inhibitor invented in our laboratory. The synthesis procedure follows a previously reported method[19]. Doxorubicin was purchased from Solarbio (Beijing, China). Scopolamine hydrobromide was obtained from Meilubio (SCOP, Dalian, China).

Preparation of F96-loaded m-PEG-PPG-PCL Polymeric Micelles F96-loaded m-PEG-PPG-PCL polymer micelles (F96-mPPP) were prepared through the self-assembly of block copolymers. Specifically, 20 mg of the lipophilic NAAA inhibitor F96 was dissolved in 500 μL of ethanol, while 200 mg of m-PPP was dissolved in a separate tube containing 1.5 mL of ethanol. The two solutions were mixed thoroughly under ultrasonication at 60 Hz and 25℃ for 10min. Subsequently, the mixed solution was slowly added and uniformly dispersed into 10 mL phosphate buffered saline (PBS, 10 mmol/L) under ultrasonication for another 10min. The solution was evaporated using a rotary evaporator at room temperature to remove the ethanol. Tween-80 was selected as a stabilizer and subsequently added to the aforementioned solution at a final concentration of 1% (w/v). The F96-loaded micelles were then centrifuged at 5000 rpm for 10min to remove any unloaded drugs and were stored at 4℃ for future use. The preparation of the corresponding empty micelles and doxorubicin-loaded micelles followed the same procedure.

Determination of the Size of F96-mPPP Micelles F96-loaded micelles were diluted with ultrapure water to a concentration of 100 μg/mL. The particle size of prepared F96-loaded micelles was measured using a laser particle size analyzer (Zetasizer Nano ZSP, Malvern Instruments, UK). Briefly, the diluted micellar solution was dropped into a clean cuvette and analyzed in the laser particle size analyzer with a grating gap set to 200 nm at 25℃. The morphological examination of the nano-micelles was carried out using a transmission electron microscope (TEM, Tecnai G2 Spirit, FEI, USA).

Evaluation of the Encapsulation Efficiency of F96-mPPP Micelles The prepared nano-sized micelles were placed into a dialysis bag (3500 D) for 6h to remove excess drugs. The collected solution was lyophilized and then re-dissolved using acetonitrile. Subsequently, the quantity of the encapsulated drug was determined using reversed-phase high-performance liquid chromatography (HPLC). NAAA inhibitor was eluted using a constant mobile phase comprising 50% acetonitrile

at a flow rate of 1 mL/min. Detection was performed at a wavelength of 210 nm, while the column temperature was maintained at 30°C.

Animals and Groups Female C57BL/6 mice aged 8–12wk were used in these experiments and were randomly assigned to one of the following 4 groups (n=8). All animals were euthanized 10d after scopolamine hydrobromide injection, and their eye tissues were collected for further analysis. The dry-eye symptoms were determined by clinical assessments as tear volumes measurement, corneal fluorescein staining and quantification of conjunctival goblet cells.

Control Group The mice were housed in standard laboratory conditions for a period of 10d.

DED Group Scopolamine hydrobromide (2.5 mg/mL) was utilized to induce dry eye symptoms based on previous protocols[20]. In brief, female mice received subcutaneous injections of 0.2 mL scopolamine hydrobromide 4 times daily (at 9 *a.m.*, 12 *p.m.*, 3 *p.m.*, and 6 *p.m.*) for 5 consecutive days. Following the injections, the mice were housed in a specifically controlled ventilated chamber at 25°C and a relative humidity below 40%. From the 5th to the 10th day, the mice were exposed to a ventilated environment without further scopolamine hydrobromide injections.

Vehicle Group Following administration of scopolamine hydrobromide for 5d, the mice were housed in the same ventilated caging. From the 5th to the 10th day, 5 μL of empty mPPP micelle eye drops (vehicle) were applied 4 times daily.

F96 Group Following the administration of scopolamine hydrobromide for 5d, the mice were housed in the same ventilated caging. From the 5th to the 10th day, 5 μL of F96 loaded mPPP micelle eye drops were applied 4 times daily.

Measurement of Tear Production Phenol red thread (PRT, Yokota, Tokyo, Japan) test was conducted to measure tear production. The cotton thread turns from yellow to red after absorbing the aqueous tears. Following the previous reports[9], the thread was positioned on the lower conjunctival fornix close to the lateral canthus at approximately 1/3 of the length of the lower lid for a duration of 15s. Subsequently, length of the red wetted portion was immediately measured in millimeters using vernier caliper.

Measurement of Corneal Permeability Corneal fluorescein staining was conducted to evaluate alterations in corneal epithelial integrity. Specifically, 0.5 mL of Oregon green dextran (OGD, 70 kDa, Invitrogen, Eugene, Oregon, USA, 50 mg/mL) was administered to the mice cornea for 1min. Subsequently, the mice were euthanized, and the eyes were washed with 1 mL of saline five times. Digital images were captured using a stereoscopic zoom microscope (AZ100, Nikon, Tokyo, Japan) with an excitation wavelength of 470 nm and an emission wavelength of 488 nm. The average fluorescence intensity of corneal OGD staining was determined for a 3-mm diameter circle in the central cornea region using analysis software (NIS Elements, version 4.1, Nikon, Melville, NY, USA).

Periodic Acid-Schiff Staining Experimental mice eyes were surgically excised and fixed in a 4% polyformaldehyde (PFA) solution for 24h. Then, the tissues were embedded in paraffin, sectioned into 5-μm slices, and stained with periodic acid, Schiff reagent, and hematoxylin successively. Goblet cells in the superior, middle, and inferior conjunctivae were quantified using a light microscope (Eclipse 50i, Nikon, Tokyo, Japan).

TUNEL Assay One of the typical symptoms of DED is corneal epithelial cell apoptosis. To assess the protective effects of F96-mPPP micelles against corneal epithelial cell apoptosis, the Terminal dUTP Nick-End Labeling (TUNEL) method was performed. Frozen-embedded sections of whole eye tissues were subjected to TUNEL staining using an *in situ* apoptosis detection kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Subsequently, the sections were counterstained with diamidino-2-phenylindole (DAPI, Beyotime, Shanghai, China).

Evaluation of Micelles Retention Time on the Ocular Surface The intrinsic fluorescence properties and ability to penetrate the nucleus of doxorubicin can be used to evaluate the retention time of micelles on the ocular surface. According to the preparation procedure for F96-mPPP, encapsulate doxorubicin into the micelle (DOX-mPPP). Prepare a 1 mg/mL doxorubicin-mPPP micelle and a PBS solution of doxorubicin (DOX-PBS) at the same concentration. Subsequently, mice were assigned to two groups and were treated with DOX-mPPP and DOX-PBS on the ocular surface. The mice were euthanized at 1min, 15min, and 4h post-treatment, and their eyeballs were rinsed with 5 mL of PBS. Subsequent to enucleation, the ocular tissues were imaged at 488 nm using a fluorescence dissecting microscope (Leica, Wetzlar, Germany).

Statistical Analysis Statistical analyses were performed using GraphPad Prism 9.5.0 software (GraphPad Software, San Diego, CA, USA). The results are expressed as mean±standard error of the mean (SEM). Statistical comparisons were assessed through unpaired t-tests or one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests. P value of <0.05 indicates statistical significance.

RESULTS

Physical Characteristics of F96-mPPP Micelles To improve the solubility and stability of the hydrophobic NAAA inhibitor F96, a biodegradable F96-mPPP polymeric micelle was prepared. The distribution of F96-mPPP micelles was uniform, with a particle size of 125±41 nm (Figure 1A). The polydispersity index (PDI) of polymer micelles is an index that characterizes the dispersibility of polymers and the

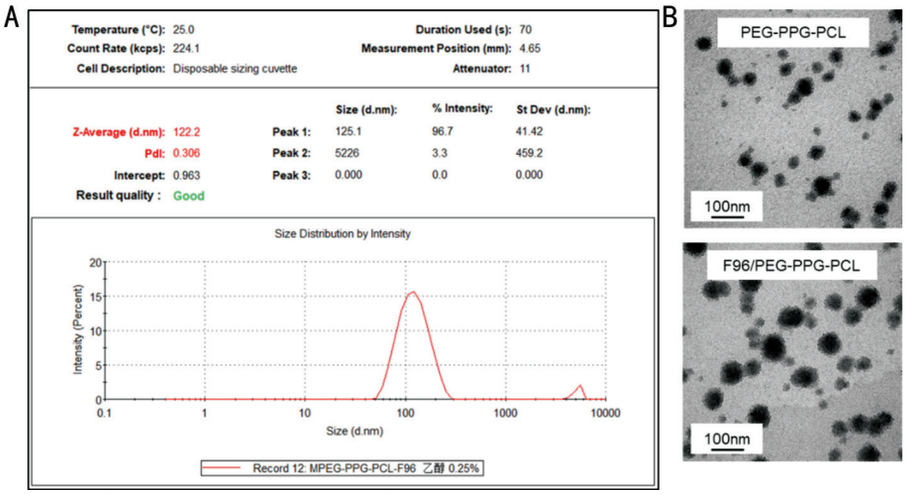

Figure 1 The morphology of F96/PEG-PPG-PCL A: The corresponding DLS results, the particle size distribution is 125±41 nm; B: the TEM image of M-PPP and F96/PEG-PPG-PCL (F96-m-PPP), F96-m-PPP micelles are uniformly dispersed microspheres. PEG-PPG-PCL: An amphiphilic multiblock copolymer, poly(ether ester urethane)s composed of poly(ethylene glycol), poly(propylene glycol), and poly(ε-caprolactone). F96-m-PPP: F96-loaded m-PEG-PPG-PCL polymer micelles.

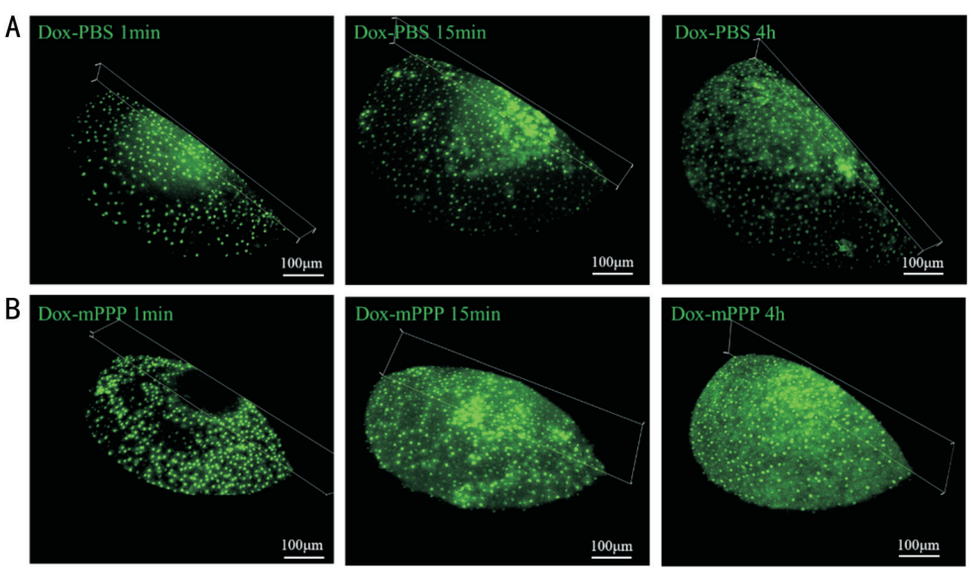

Figure 2 The retention and permeability of Dox-mPPP and Dox-PBS in cornea A: Dox-PBS retention from 1min to 4h on ocular surface, scale bar, 100 μm; B: Dox-mPPP retention from 1min to 4h on ocular surface. Dox-mPPP: 1 mg/mL doxorubicin-mPPP micelle; PBS: Phosphate buffered saline; Dox-PBS: 1 mg/mL PBS solution of doxorubicin.

particle size distribution. The PDI value of F96-mPPP nano-micelle eye drops was 0.306 (Figure 1A), which indicated that the distribution range of micelles was concentrated with an excellent micelle quality.

The TEM results revealed the uniform dispersion of F96-loaded m-PPP micelles in a spherical form (Figure 1B). A significant increase in the particle size of the drug-loaded sphere was observed compared to the empty carrier m-PPP micelles, indicating effective encapsulation of NAAA inhibitors in the micelles. The percentage of F96 encapsulation was determined using the formula: EE%=(1−Cf/Ct)×100%, where Cf represents the amount of free drug and Ct is the mixture of drugs in the micelle suspension. The encapsulation rate of F96-mPPP nano-micelle eye drops was calculated to be 94%.

m-PPP increased Drug Retention time and Permeability in Cornea Doxorubicin was utilized as a fluorescent indicator of liposomal structure to evaluate the trans-corneal penetration of multiblock polymer m-PPP. DOX-mPPP micelles and DOX-PBS solutions were both prepared at a concentration of 1 mg/mL and were topically applied on the ocular surface of mice. The experimental mice were then sacrificed at predetermined time points, and examined using a microscopy (Figure 2). There was no significant difference in the fluorescence intensity between DOX-PBS and DOX-mPPP groups at the initial 1min following eye drops application. For the DOX-PBS groups, rapid precorneal clearance, attributed to lid blinking and tear production, limited the exposure of the fluorescent agents, resulting in a similar fraction of absorbed dose between the

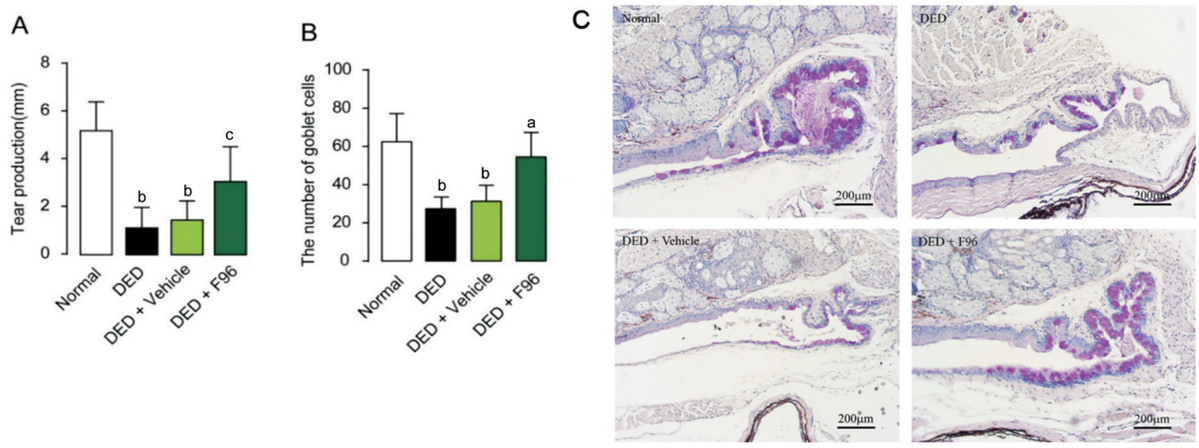

Figure 3 Tear production and number of goblet cells in dry eye mice A: Quantification of tear secretion measured by phenolic cotton wool line; B: Quantification of goblet cells number; C: Periodic acid-Schiff staining of conjunctiva tissue of normal, DED or DED mice on day 10 after repeated treatment of vehicle or F96 eye drops. Scale bars, 200 μm. [a]*P*<0.01 *vs* DED+vehicle; [b]*P*<0.001 *vs* normal; [c]*P*<0.001 *vs* DED+vehicle; *n*=8. DED: Dry eye disease; Normal: Control group; DED+Vehicle: Vehicle group; DED+F96: F96 group.

1-min and 15-min groups. However, in the 15-min treatment groups, the corneal topical fluorescence intensity of DOX-mPPP was greatly higher than the corresponding DOX-PBS group, which could sustain for a minimum of 4h. These experimental results indicated that the polymer vector mPPP prolonged the precorneal residence time, thereby increasing the corneal contact amount of nano-micelle-encapsulated DOX and improving ocular bioavailability of topical eye drops.

F96-mPPP Preserved Tear Secretion and the Quantity of Conjunctival Goblet Cells Diminished tear production leads to tear hyperosmolarity, which is identified as the primary mechanism responsible for inflammation, damage, and dry eye symptoms on the ocular surface. In this study, the phenol red thread test was employed to assess tear production. There was a significant decrease in phenol thread wetting portion following the administration of scopolamine (5.15±0.60 mm for the control group *vs* 1.46±0.30 mm for the DED group, *P*<0.001; Figure 3A), indicating a notable reduction in tear production in DED mice. Treatment with F96-mPPP nano-micelle eye drops resulted in a more pronounced increase in phenol thread wetting compared to the application of the vehicle m-PPP group (1.85±0.51 mm for the vehicle group *vs* 3.35±0.45 mm for the F96 group, *P*<0.001; Figure 3A). These findings suggested that the F96-loaded polymer micelles enhanced tear production during the scopolamine-induced DED progression.

Tear hyperosmolarity has been identified to be responsible for the reduction in conjunctival goblet cell density, a common occurrence in ocular surface of DED. Goblet cells located in the interpalpebral bulbar conjunctiva play a crucial role in secreting gel-forming mucins that are essential for maintaining the stability of the tear film and the homeostasis of the ocular surface. In this study, the density of goblet cells was assessed using periodic acid-Schiff (PAS) staining. Scopolamine-induced DED resulted in significant atrophy in goblet cells as well as a noticeable reduction in their numbers (27.4±2.7 for the DED group *vs* 62.4±5.6 for the control group, *P*<0.001; Figure 3B, 3C) compared to the control group. Treatment with F96-mPPP nano-micelle eye drops for 5d resulted in a marked increase in conjunctival goblet cell density (54.5±4.5 for the F96 group *vs* 31.3±3.0 for the vehicle group, *P*<0.01; Figure 3B, 3C) and maintained their normal morphology. Conversely, the use of the empty m-PPP carrier did not ameliorate the pathological alterations induced by DED.

F96-mPPP Prevented Corneal Barrier Function Damage Increased permeability of fluorescein dyes in the cornea is believed to be positively associated with corneal injury in DED patients. As a result, the fluorescence intensity is used to indicate corneal epithelial injury and estimate the therapeutic effects for dry eye treatment. In this experiment, the fluorescent agent OGD was used to evaluate corneal barrier impairment in DED mice. The average OGD staining score was calculated according to Baylor grading scheme (Figure 4F). Analysis of OGD data revealed that the uptake of OGD and scores for corneal fluorescein staining were significantly higher in the DED group than in the control group (7.7±0.72 for DED group *vs* 2.1±0.31 for control group, *P*<0.001; Figure 4A, 4B). These results indicated a notable increase in corneal barrier disruption and damage in mice with DED. Treatment with m-PPP alone did not ameliorate the pathological conditions; however, treatment with F96-loaded nano-micelles led to a significant reduction in ocular surface fluorescence intensity (6.5±0.72 for vehicle group *vs* 3.4±0.32 for F96 treatment group, *P*<0.001; Figure 4C–4E).

F96-mPPP Down-Regulated Apoptosis in Corneal Epithelium Clinical observations have confirmed a significant

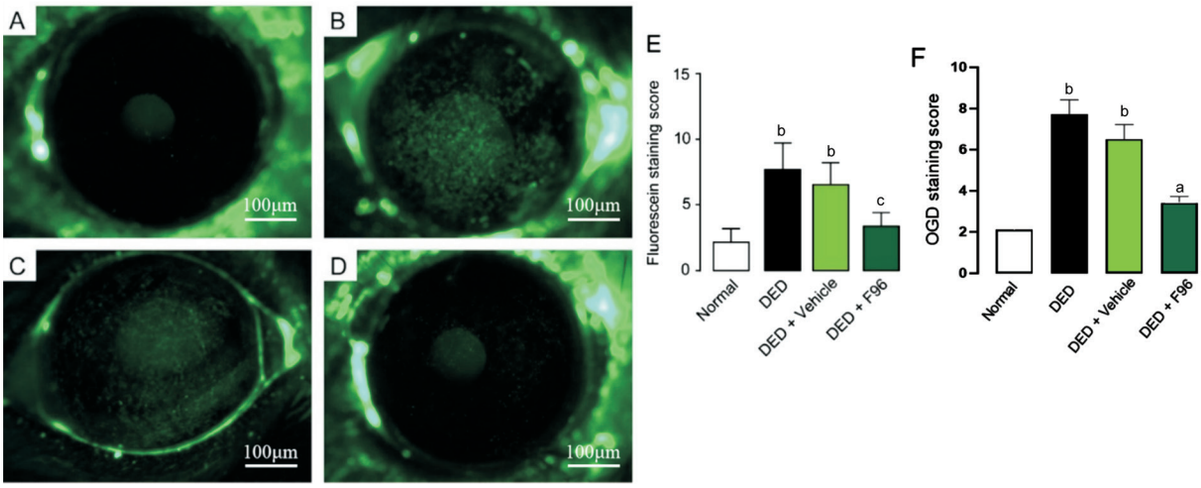

Figure 4 Corneal damage measured with OGD staining in DED mice A: OGD staining of cornea in normal mice; B: DED mice; C: DED mice under treatment with vehicle; D: F96 eye drops, E: OGD fluorescence staining score of DED mice; F: Ocular surface integrity was evaluated through OGD staining. Scale bars, 100 μm. Fluorescent analyses and quantification of OGD staining; [a]*P*<0.01 *vs* DED+vehicle; [b]*P*<0.001 *vs* normal; [c]*P*<0.001 *vs* DED+vehicle; *n*=8. OGD: Oregon green dextran; DED: Dry eye disease; Normal: Control group; DED+Vehicle: Vehicle group; DED+F96: F96 group.

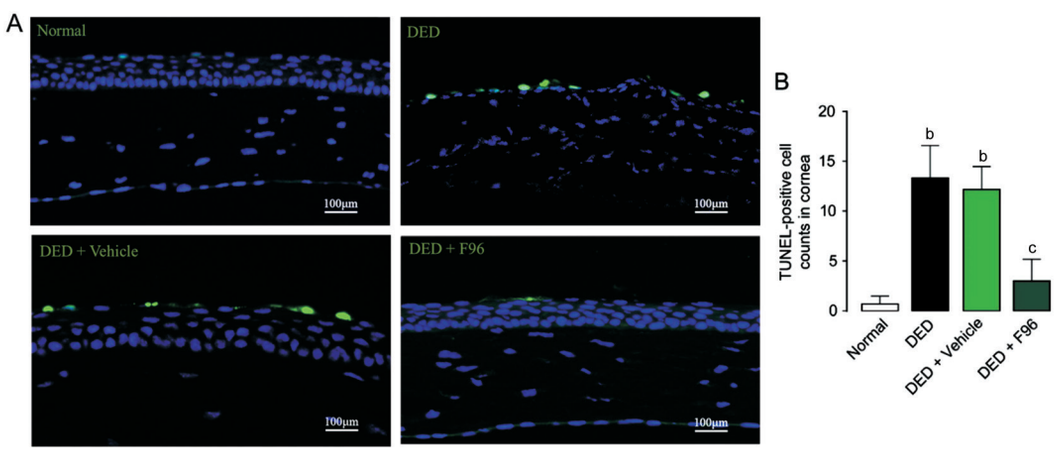

Figure 5 F96 inhibited apoptosis in dry eye corneas A: The positive cell in the corneal epithelial cell were examined by TUNEL staining on Day 10; B: The estimated representation of the TUNEL-positive cell number per corneal section. [b]*P*<0.001 *vs* normal; [c]*P*<0.001 *vs* DED+vehicle. DED: Dry eye disease; Normal: Control group; DED+Vehicle: Vehicle group; DED+F96: F96 group; TUNEL: Terminal dUTP nick-end labeling.

increase in apoptosis levels in the corneal epithelium of DED patients compared to those with normal eyes. Here, TUNEL positive cells were counted in the corneal sagittal sections to assess the apoptotic cells on the ocular surface by fluorescence microscope. The number of TUNEL-positive cells in the DED group was markedly higher compared to the normal group (Figure 5). Interestingly, there was no difference between the DED group and the vehicle group, suggesting that m-PPP did not have a therapeutic effect on DED. However, the number of TUNEL-positive cells decreased notably in mice treated with F96-mPPP eye drops (Figure 5). This finding indicates that F96 may have a significant therapeutic effect in restoring the corneal barrier.

DISCUSSION

Despite the growing clinical interest in DED, the effective treatments for this disease remain limited. Artificial tear drops, the primary treatment for DED, are only temporary effective to mild and moderate patients with requirements of repeated instillations[21]. Anti-inflammatory agents and immunosuppressants, such as corticosteroids and CyA, have shown potential in alleviating clinical symptoms of severe DED patients[21-22]. However, long term use of these compounds is associated with numerous adverse effects. For example, long-term use of corticosteroids poses risks of cardiovascular toxicity, gastrointestinal ulceration, and increased intraocular pressure[23-24]. However, short-term use of CyA may mainly cause a burning and stinging sensation in the eyes[25-27], while long-term use may be accompanied by risks such as epithelial keratopathy, renal toxicity, and abnormal liver function[28-30]. It is realized that new approach needs to be found for DED drugs research and development without inducing severe side effects. Autacoids are chemical messengers that influence a number of

pathways without inducing side effects, which is particularly suitable for addressing multitarget diseases like DED. PEA belongs to a kind of lipid autacoids which is usually produced by local mast cells, microglia, and macrophages, exerting specific biological effects in the immediate microenvironment. Previous reports highlighted the beneficial effects of PEA in several ocular diseases, thus, preserving the local PEA levels by inhibiting its degradation is also considered a promising strategy for treating DED.

The corneal epithelium and endothelium restrict the entry of hydrophilic molecules into the aqueous humor, but facilitate the passage of small lipophilic molecules[31]. On the contrary, the corneal stroma prevents the passage of lipophilic molecules, while allows the diffusion of hydrophilic agents[32]. This distinctive structure of ocular surface leads to a unique barrier, limiting the diffusion and penetration of most lipophilic and hydrophilic drugs into the eyes. Therefore, in the present study, an amphiphilic multiblock poly (ether ester urethane)s comprising m-PPP was selected for the preparation of the polymeric micelles.

Amphiphilic polymers are utilized to solubilize hydrophobic compounds in eye drop formulations for the treatment of ocular surface disease. The incorporation of both hydrophilic and hydrophobic segments enables the polymer to dissolve in both the corneal epithelium and stroma simultaneously, thereby prolonging the retention time on ocular surface and improving bioavailability of ophthalmic formulation. In the present polymer micelle, PPG was selected as a hydrophobic inner core to encapsulate fat-soluble bioactive agents due to its relatively high stability. PEG is a kind of water-soluble nonionic polymers that widely used for drugs delivery and self-assembled micelles preparation. The addition of PEG to the micelles resulted in the formation of a hydrophilic outer shell surrounding the drug-loaded inner core, leading to improved stability in precorneal fluid. However, a major limitation of PEG-PPG deblock copolymers is nonbiodegradability, which hindered controlled drug release. To address this issue, PCL, a biocompatible polyester, was introduced to fabricate the biodegradable polymer micelles. PCL has been the main component of surgical sutures and implantable contraceptive device approved by the Food and Drug Administration (FDA), which degraded into 6-hydroxyhexanoic acid, a safe naturally occurring human metabolite[33]. Poly(ether ester urethane)s scaffolds containing PEG, PPG, and PCL segments provided enhanced abilities to increase the solubility and penetration hydrophobic drug, as well as improved drug release profiles. The experimental results revealed promising novel applications of mPPP-NAAA inhibitors in the treatment of DED.

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