• Basic Research •

Hydrogel dressings on neurotrophic keratitis in an experimental animal model

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Abstract

● **AIM:** To investigate the therapeutic effects of hydrogel dressings on neurotrophic keratitis in rats.

● **METHODS:** Male Wistar rats, aged 42-56d, were randomly divided into control, experimental, and treatment groups, each consisting of five rats. The experimental and treatment groups underwent neurotrophic keratitis modeling in both eyes. After successful modeling, biomedical hydrogels formed with polyvinyl alcohol and polyvinyl pyrrolidone were used in treatment group for 7d. Ocular irritation response and keratitis index scores, Schirmer's test, tear film break-up time (BUT), sodium fluorescein staining, and hematoxylin and eosin (HE) staining were used to evaluate the effectiveness of the treatment.

● **RESULTS:** The neurotrophic keratitis model was successfully established in rats with severe ophthalmic nerve injury, characterized by keratitis, ocular irritation, reduced tear secretion measured by decreased BUT and Schirmer test values, corneal epithelial loss, and disorganized collagen fibers in the stromal layer. Following treatment with hydrogel dressings, significant improvements were observed in keratitis scores and ocular irritation symptoms in model eyes. Although the recovery of tear secretion, as measured by the Schirmer's test, did not show statistical differences, BUT was significantly prolonged. Fluorescein staining confirmed a reduction in the extent of corneal epithelial loss after treatment. HE staining revealed the restoration of the structural disorder in both the epithelial and stromal layers to a certain extent.

● **CONCLUSION:** Hydrogel dressing reduces ocular surface irritation, improves tear film stability, and promotes the repair and restoration of damaged epithelial cells by maintaining a moist and clean environment on the ocular surface in the rat model.

● **KEYWORDS:** neurotrophic keratitis; hydrogel; corneal epithelial cells; rat
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INTRODUCTION

Neurotrophic keratitis is a degenerative corneal disease characterized by a decrease in or absence of corneal perception[1]. This condition occurs primarily due to partial or complete damage to the trigeminal nerve. Corneal perception is crucial for maintaining optimal corneal function. It facilitates tear secretion through the ocular irritation reflex, thereby preserving ocular surface moisture[2]. Moreover, corneal nerves enable the production of neurotrophic factors that nourish corneal tissues and facilitate the healing of corneal damage[3-4]. When the trigeminal nerve sustains an injury, corneal perception diminishes, leading to reduced tear secretion, diminished eyeblink reflex, and a decline in the production of neurotrophic factors. Consequently, damage to the corneal epithelium occurs, resulting in delayed healing, and potentially leading to corneal ulcers and perforations. Ultimately, this severely impairs visual acuity.

Hydrogels are highly hydrophilic with a three-dimensional network structure that can absorb a significant volume of water while maintaining its integrity[5]. Medical hydrogels constructed from carefully selected materials demonstrate excellent histocompatibility and have been extensively used in the management of skin wounds. They have been demonstrated to effectively maintain the wound moisture, prevent infections, and promote wound healing[6-8]. The use of hydrogel materials has steadily increased in the field of ophthalmology. These materials have applications in contact lenses and hydrogel-containing drugs. For example, silicone

hydrogel contact lenses have been scientifically validated owing to their excellent wettability and patient comfort[9]. Furthermore, in the domain of hydrogel-containing drugs, hydrogel-containing dexamethasone/netilmicin has been substantiated for its efficacy in mitigating inflammation after cataract surgery with a lower medication frequency than traditional eye drops, thereby enhancing patient compliance[10]. However, the application of hydrogels in neurotrophic keratitis remains relatively limited, necessitating expansion of their potential applications in this domain.

MATERIALS AND METHODS

Ethical Approval This study was conducted in accordance with the ethical guidelines of the Institutional Review Board of Peking University Third Hospital (A2023027). The study was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Induction of Neurotrophic Keratitis in Animal Model Fifteen male Wistar rats, aged 42–56d and meeting the SPF grade criteria, were procured from the Hubei Provincial Laboratory Animal Research Center. The rats were housed in a controlled environment with a relative humidity of 50%–60% and a temperature of 24.0℃±1.0℃. Subsequently, the rats were randomly divided into three groups: control, experimental, and treatment, each consisting of five rats. The control group received no modeling or treatment, whereas the experimental and treatment groups received induction of neurotrophic keratitis in both eyes. Following successful modeling, the treatment group received application of BURN CARING HD-N hydrogel (JA Biotech Co. Ltd., Changchun, China). The rats were allowed 7d of acclimatization upon arrival before undergoing neurotrophic keratitis modeling.

During the experiment, the rats were anesthetized and placed in prone position. The hair surrounding the eyes was carefully removed and a cut was made along the temporal conjunctival vault to expose the posterior visual field of the eye. The eyeball was gently turned towards the nasal side using a fixator to facilitate access to the ophthalmic branch of the trigeminal nerve. The ophthalmic nerve was identified and clipped to ensure that no bleeding occurred at the surgical site (Figure 1). Following this procedure, the rats were allowed to recover and were returned to their cages. As a precautionary measure, all rats received prophylactic levofloxacin eye drops daily for a week. Once corneal keratitis was observed, hydrogel treatment was initiated. The hydrogel was applied to the eyes of the rats for 6h daily for 7d (Figure 2), followed by subsequent examinations, as described below.

Hydrogel The hydrogel material used in this study was fabricated using a polyvinyl alcohol-polyvinylpyrrolidone (PVA-PVP) hydrogel system. PVA and PVP are crosslinked to construct a three-dimensional network structure under

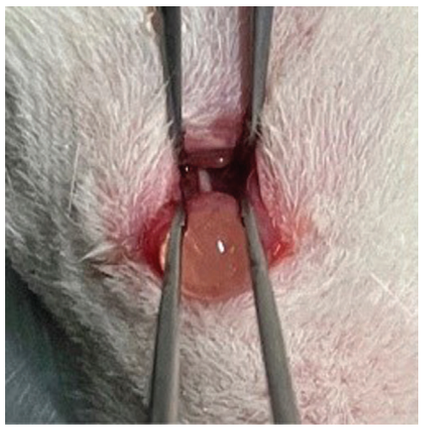

Figure 1 Process of ophthalmic nerve clipping.

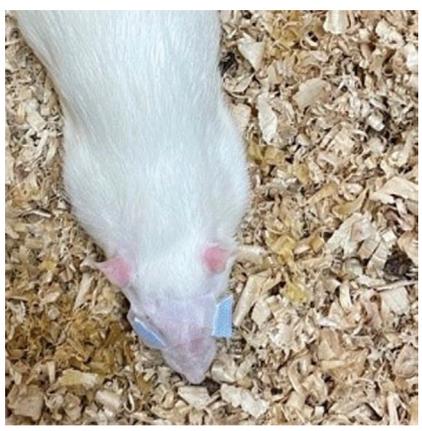

Figure 2 Image of hydrogel dressing on day 1 The hydrogel material was cut into appropriate sizes to cover the mouse cornea, secured with tape on both eyes of the mouse, and applied for 6h daily for 7d.

irradiation, resulting in the formation of biocompatible hydrogel materials. These hydrogels are produced via irradiation, eliminating the need for chemical crosslinking agents and demonstrating robust safety profiles and nonallergenic properties. This material enables sustained and gradual release of moisture, thereby regulating the wound environment to facilitate repair. We identified previous studies that applied similar hydrogel materials in protecting non-surgical eye in retinal surgery, which prevents discomfort in the healthy eye postoperatively and also significantly improves the comfort of the patient's healthy eye, facilitating better cooperation with the surgeon and ensuring the smooth progress of the surgery. Thus we selecting them for further investigation in the treatment of neurotrophic keratitis[11].

Keratitis-Related Score Ocular irritation response scores and keratitis index scores were obtained at three time points before modeling[12], after modeling, and after treatment in the three groups of rats.

Schirmer's Test The wetting length of the test paper was measured in a quiet and dimly lit environment, without the use of local anesthesia. The test paper was cut into dimensions of 1×17 mm and placed in the inner corner of the rat's lower eyelid. After 1min, the length of the wet portion of the test

Table 1 Keratitis related score

Parameters	Before modeling	After modeling	After treatment
Keratitis index scores			
Control	0	0	0
Experimental	0	11.8±0.7	12.2±0.4
Treatment	0	11.6±1.0	7.6±1.0
Ocular irritation response scores			
Control	0	0	0
Experimental	0	12.2±1.5	10.0±1.1
Treatment	0	12.6±0.8	5.4±1.4

paper was measured as an indicator of the tear film stability and moisture.

Tear Film Break-up Time The tear film break-up time (BUT) was measured under controlled room temperature and humidity conditions in a room protected from light. One drop of 1% sodium fluorescein solution was gently instilled into the conjunctival sac of the rats using a sterilized dropper. The rats were then allowed to blink naturally to ensure the distribution of fluorescein across the ocular surface. The time from the last blink to the appearance of the first black spot on the cornea was recorded as tear film rupture time. This measurement was performed three times for each rat, and the average value was calculated for the analysis.

Sodium Fluorescein Staining To assess the extent of corneal damage and staining, a glass rod with one end dipped in a small amount of 1% fluorescein sodium solution was gently placed in the conjunctival sac. After allowing 1–2min for the fluorescein to distribute, a few drops of sterile saline were used to rinse the conjunctival sac. The corneas were observed under a slit lamp for staining. A 12-point scoring system was utilized for fluorescein staining evaluation. The cornea was divided into four quadrants and each quadrant was assigned a score ranging from 0 to 3 points. A score of 0 indicated no staining, 1 point represented punctate staining with 1 to 30 spots, 2 points indicated punctate staining with more than 30 spots but without fusion, and 3 points represented the presence of fused punctate staining, filaments, or ulcers in the cornea.

Hematoxylin and Eosin Staining After 7d of treatment and the corresponding examinations, the rats were euthanized in three groups. The eyeballs were carefully removed using ophthalmic scissors and washed with a saline solution. Subsequently, the eyeballs were placed in Davidson's fixative for 24h at room temperature to undergo fixation. After fixation, the eyeballs were removed from the fixative and dehydrated at room temperature. The samples were sequentially immersed in ethanol solutions with volume fractions of 70%, 80%, 90%, and 95%. Subsequently, the eyeballs were immersed in anhydrous ethanol three times, with each immersion lasting 1h. The next step involved infiltration of the dehydrated eyeballs. They were then immersed in anhydrous ethanol three times for 1h each time. They were immersed in xylene twice, with each immersion lasting 40min. Finally, the eyeballs were infiltrated with paraffin wax at a temperature of 62°C. This process was performed three times, with the first immersion lasting 30min and subsequent immersions lasting 1h each. After the infiltration step, the eyeballs were adjusted to the correct positions based on the anterior and posterior diameters. They were then embedded in hard wax at a temperature of 58°C–60°C. After cooling, the wax blocks containing eyeballs were sliced using a rotary slicer. The resulting slices had a thickness of 4 μm. The slices were subjected to a series of procedures, including water bath and spreading at a temperature of 40°C–46°C, followed by baking at 60°C for 2h. Finally, the slices were stained with hematoxylin and eosin (HE) and sealed with neutral gum. The cells were then observed under a microscope for further analysis.

Statistical Analysis All statistical analyses were performed using IBM SPSS for Mac version 26.0 (IBM Corp., Armonk, NY, USA). Independent *t*-tests were performed if they exhibited homogeneity of variance according to Levene's test to explore the differences in scores, break-up time (BUT), and Schirmer's test between the different groups. $P<0.05$ was considered statistically significant.

RESULTS

Hydrogel Relieve of Keratitis Related Score The keratitis index and eye irritation reactions in rats were scored at three different time points: before modeling, after modeling, and after treatment (Table 1). Prior to the modeling procedure, no signs of keratitis or eye irritation were observed in any rat. After modeling, both experimental and treatment groups showed more severe keratitis and eye irritation. After treatment, rats in the treatment group showed a significant reduction in the keratitis index score ($P<0.001$) and eye irritation reaction score ($P=0.001$).

Hydrogel Improve the Tear Film Stability Schirmer's test and BUT were performed for each group of rats, and the results are shown in Table 2 and Figure 3. Compared with the control group, the experimental group showed a decrease

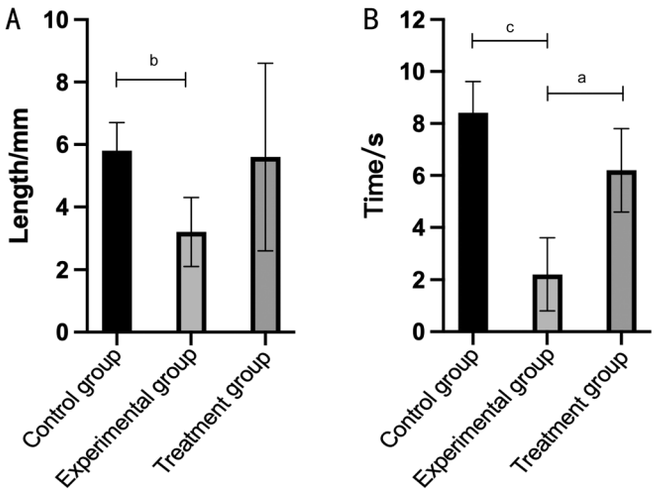

Figure 3 Results of tear related test A: Schirmer's test; B: BUT examination. BUT: Break-up time. [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001.

Table 2 Tear related test

Group	Schirmer's test, mm	BUT, s
Control	5.8±0.9	8.4±1.2
Experimental	3.2±1.1	2.2±1.4
Treatment	5.6±3.0	6.2±1.6

BUT: Break-up time.

in tear secretion, which was manifested by a decrease in the wetting length of Schirmer's test paper (*P*=0.003). The average length of the Schirmer's test increased after treatment, but did not show a statistically significant difference due to the large individual differences (*P*=0.136). As for tear film rupture time, BUT significantly decreased in the experimental group (*P*<0.001), whereas it was significantly restored after treatment (*P*=0.014).

Hydrogel Relieved the Damage of the Epithelial In the control group, the corneal epithelium did not exhibit noticeable spot staining. However, in the experimental group, the corneal epithelium displayed diffuse staining with fluorescein, appearing as dots or large flakes. The degree of corneal damage was significantly higher in the experimental group than in the control group (*P*<0.001). Although the corneal epithelium was stained in the treatment group, the degree of staining was significantly lower than that in the experimental group (*P*=0.005; Figures 4 and 5).

Hydrogel Restore the Structure of Epithelial As shown in Figure 6, in the control group, the corneal epithelial cells were well arranged, the epithelium remained intact without detachment, and the stromal collagen exhibited a regular arrangement. No obvious abnormalities were observed in the cornea. In contrast, the corneas in the experimental group appeared thinner, with visible epithelial detachment. The arrangement of the epithelial cells was disorganized, and a decrease in the number of cell layers was noted. Furthermore, collagen fibers in the stromal layer were loosely arranged and disorganized. An increase in the gap was observed between the fibrous plate laminae, and some fibers showed signs of curling, coiling, and breakage. However, the corneas in the treatment group showed signs of restoration. The relative corneal thickness improved, and the epithelium exhibited thickening with no detachment. The arrangement of the stromal layer fibers was more regular than that in the model group. The gap between the fibrous plates decreased, and the degree of lesions reduced.

DISCUSSION

This study showed that rats with severe ophthalmic nerve injury developed neurotrophic keratitis characterized by keratitis, ocular irritation, reduced tear secretion (measured by decreased BUT and Schirmer's test values), corneal epithelial loss, and disorganized collagen fibers in the stromal layer. However, following treatment with hydrogel dressings, significant improvements were observed in the keratitis scores and ocular irritation symptoms in rat eyes. While the recovery of tear secretion, as measured by the Schirmer's test, did not show statistical differences, BUT was significantly prolonged, indicating the restoration of tear quality. Fluorescein staining confirmed a reduction in the extent of corneal epithelial loss after treatment. HE staining revealed the restoration of the structural disorder in both the epithelial and stromal layers to a certain extent.

Neurotrophic keratitis is a degenerative corneal disease caused by an ophthalmic nerve injury. It can lead to corneal epithelial damage, ulceration, and perforation due to the absence of corneal perception and neurotrophic factors[1]. Animal models of neurotrophic keratitis exhibit swollen corneal epithelial cells, absence of microvilli, and an abnormal basement membrane[13]. Delayed wound healing and excessive epithelial cell shedding occur in albino rabbits with neurotrophic keratitis, resulting in persistent epithelial cell loss[14]. Previous studies have proposed several potential mechanisms, including reduced tear production due to corneal hyperalgesia[15], dryness of the ocular surface due to reduced reflexes, and a decrease in neurotrophins on the ocular surface[16], particularly nerve growth factors (NGF). NGF plays a crucial role in the proliferation, differentiation, healing, and remodeling of corneal epithelial cells. These mechanisms contribute to decreased tear secretion, thinning of the tear film, and impaired proliferation and differentiation of corneal epithelial cells, resulting in long-term damage to the corneal epithelium, exposure of the stroma, stromal dissolution[17], and eventual corneal perforation[18]. Thus, early treatment is important to prevent further progression of neurotrophic keratitis.

Treatment options for neurotrophic keratitis can be divided into nonsurgical and surgical treatments depending on the severity of the condition. In cases in which corneal involvement is limited to the epithelium, the primary goals of treatment are to maintain the stability of the epithelium,

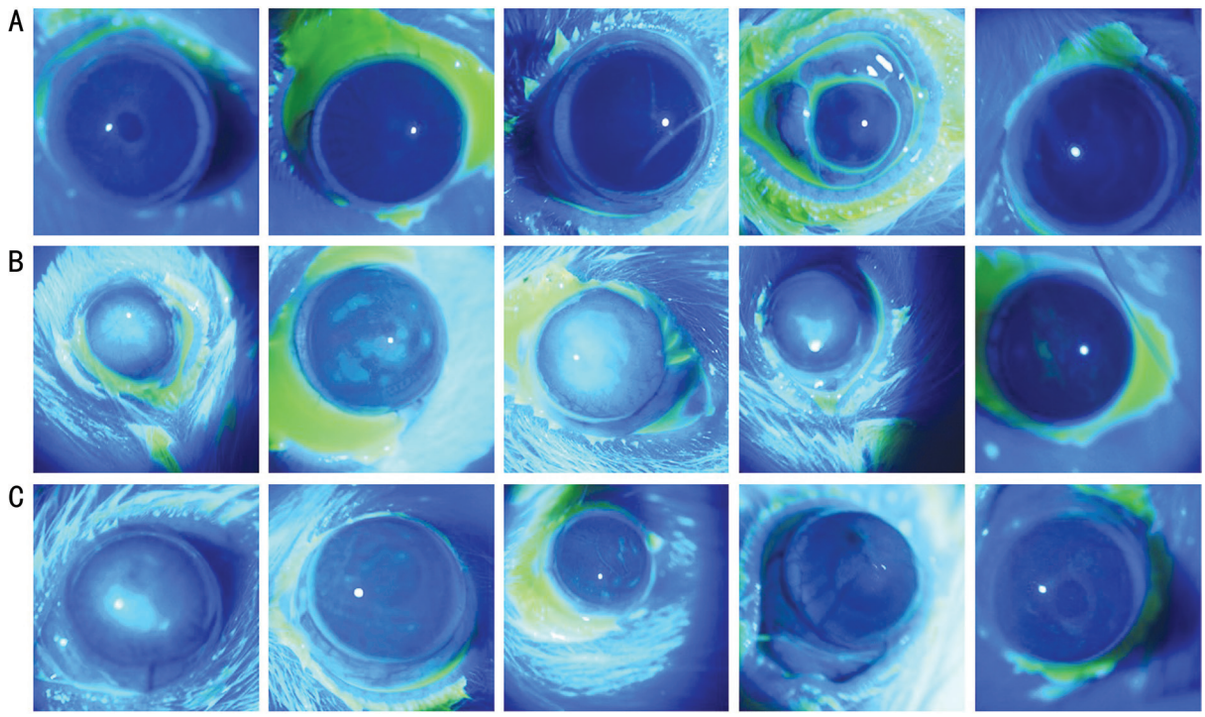

Figure 4 Sodium fluorescein staining of cornea A: Control group; B: Experimental group; C: Treatment group.

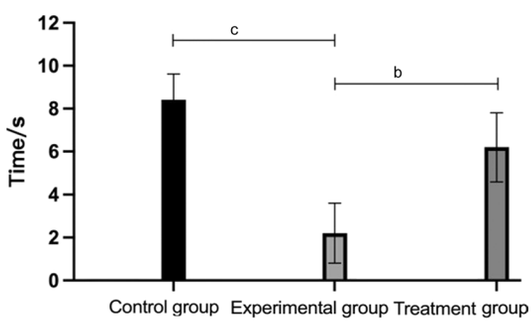

Figure 5 Results of sodium fluorescein staining score $^{b}P<0.01$, $^{c}P<0.001$.

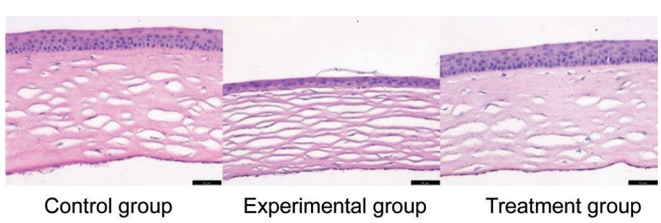

Figure 6 Results of HE staining of cornea HE: Hematoxylin and eosin. Scale bar: 50 μm.

promote healing of damaged epithelial cells, and prevent progression of the lesion into the stroma. Use of unpreserved lubricants is recommended to moisturize the ocular surface because preservatives can have toxic effects[19]. Anti-infectious and anti-inflammatory medications may also be prescribed if an infection or inflammation is observed. When the stroma is involved, serum eye drops can be administered to expedite epithelial healing[20]. Additionally, nonsurgical methods, such as eyelid closure or therapeutic contact lenses, can be employed. In cases of corneal perforation, options such as amniotic membrane or corneal transplantation may be considered to save the eyeball. Recently, various biological and medical products have emerged as potential treatment options. For example, recombinant human NGF can be used to supplement endogenous NGF deficiency caused by corneal nerve damage[21-23]. However, the current treatment regimens often involve multiple medications and frequent applications, which can lead to poor patient compliance.

Hydrogels are three-dimensional networks of cross-linked polymers that can absorb and retain large amounts of liquid. They have gained significant attention and have been extensively used in various applications, such as cell culture, drug delivery, wound dressings, and tissue engineering[5]. A key advantage of hydrogels is their high-water content, which allows them to closely resemble living tissues. Their soft and porous structures render them suitable for creating moist environments that promotes wound healing[7]. In wound-healing applications, hydrogels can form a physical barrier on the wound surface, absorb excess secretions, and provide an optimal environment for tissue repair[6,8]. They can also be used to fill irregularly shaped wounds. Recently, multifunctional hydrogel materials that incorporate specific active molecules have been developed. These materials exhibit antimicrobial and blood coagulation properties, further enhancing their potential for wound healing applications[24]. In ophthalmology, hydrogel materials are used to fabricate silicone-hydrogel contact lenses. These lenses have significantly improved oxygen permeability compared to traditional lenses, allowing for longer and more comfortable wear[25-26]. In terms of corneal injury, studies

have also been conducted on the application of hydrogels. For example, the commercial hydrogel product, ReSure Sealant, has been approved by the FDA for closing corneal surgery incisions. Compared to traditional sutures, the immediate use of ReSure Sealant on corneal incisions after surgery can promote closure of the incision, reduce astigmatism, and alleviate patient discomfort[27]. Hydrogels have also been used for corneal transplantation. Zhao *et al*[28] applied a gelatin methacrylate-based hydrogel sealant to cover wounds after corneal transplantation, while incorporating oxidized dextran to strengthen adhesiveness. It was found that 56d postoperatively, the corneal graft and bed were well apposed, and the graft achieved satisfactory re-epithelialization. These results indicate that hydrogels have great potential in ophthalmology, especially in the field of corneal diseases.

In this study, a hydrogel material was applied to treat neurotrophic keratitis in rats. Preliminary results indicated that the hydrogel material effectively alleviated the ocular signs associated with neurotrophic keratitis. Various indicators were evaluated to assess the treatment efficacy. The keratitis and ocular irritation index scores confirmed that hydrogel treatment significantly relieved symptoms such as conjunctival congestion, edema, photophobia, and reduced the corneal lesion area. Tear-related tests demonstrated improved tear film stability and, to some extent, tear secretion. Morphological tests revealed a reduction in corneal epithelial cell loss, restoration of the number of epithelial cell layers, and an improvement in stromal structural disturbances. Neurotrophic keratitis involves two main aspects of its pathogenesis: reduced ocular surface wetness and a lack of neurotrophic factors. Previous studies have reported that the primary mechanism of action of hydrogel materials in treating corneal diseases is to serve as artificial corneal substitutes to partially restore corneal function, or to load drugs as sustained drug delivery systems[29]. In this study, the hydrogel dressing treatment was primarily focused on maintaining a moist environment on the ocular surface. The loose and porous nature of hydrogels allows them to absorb large amounts of water, thereby preserving the moist state of the ocular surface. This helps prevent damage and detachment of the corneal epithelium caused by reduced tear secretion and nerve reflexes. Additionally, a moist environment supports the repair of damaged corneal epithelial cells. The relevant mechanisms have been proposed in previous studies. One study found that in an alkali-burned rabbit eye model, hydrogels covering the limbal stem cells promoted epithelial remodeling[30]. *In vitro* studies have also demonstrated that hydrogels can promote the differentiation of corneal stromal stem cells into corneal cells, producing a sufficient amount of extracellular matrix to repair corneal damage[29]. Furthermore, one of the factors contributing to the deterioration of neurotrophic keratitis is secondary infection due to epithelial defects. Hydrogel materials can act as barriers preventing the invasion of pathogens and reducing the likelihood of infection. Previous studies have also indicated that hydrogel materials without drugs can promote the repair of corneal epithelial injury. The main mechanism is their ability to form a protective layer on the damaged corneal surface, thereby improving comfort and promoting the repair of the corneal epithelium, while stabilizing the microenvironment of the ocular surface to create conditions more conducive to recovery[31]. In future, researchers should consider incorporating neurotrophic factors such as NGF into hydrogel materials to address the disease from another pathogenic perspective and potentially achieve improved efficacy. Previous studies have provided precedents wherein the addition of β-1,3-glucan (a wound healing agent) and SB431542 (an antiscarring agent) to hydrogel materials has been shown to better promote the repair of corneal injuries[32]. However, further investigations are required to fully understand the potential benefits and optimize the treatment approach for neurotrophic keratitis.

This study had some limitations. First, the sample size was relatively small, and should be increased to make the conclusions more robust. In addition, neurotrophic factors, such as NGF, in the cornea before and after treatment were not examined; therefore, it was not possible to confirm whether the hydrogel dressing could restore the level of neurotrophic factors. Moreover, the study used only a mild neurotrophic keratitis model and did not observe the efficacy of neurotrophic keratitis involving the stroma. The next step of the experiment should be to expand the sample size, observe the efficacy of different degrees of neurotrophic keratitis, and add a control group of artificial tears in order to clarify the efficacy of the hydrogel compared with the current treatment.

In conclusion, the application of hydrogel dressings in rats with neurotrophic keratitis showed promising results in reducing ocular surface irritation, improving tear film stability, and promoting the repair and restoration of damaged epithelial cells by maintaining a moist and clean environment on the ocular surface.

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Authors' contributions: Xia HQ, Jiang XD, and Tian YT designed the research; Xia HQ, Jiang XD, and Song YF performed the study; Xia HQ, Jiang XD performed the statistical analysis; Xia HQ and Jiang XD drafted the manuscript; Li XM and Tian YJ revised the manuscript; Li XM and Tian YJ are responsible for the overall content as guarantor. All authors reviewed the manuscript.

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