Preparation of Thermosensitive Anti-inflammatory Lacrimal Plugs and Observation of Their Effects in Dry Eye Treatment

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Abstract: This study aims to develop a novel thermosensitive lacrimal plug material to address current challenges in dry eye treatment. We prepared thermosensitive lacrimal plugs using N-isopropylacrylamide (NIPAM) hydrogel, loaded with dexamethasone and mitomycin C to impart anti-inflammatory and anti-fibrotic properties to the material. Rheological tests demonstrated that the material exhibits good fluidity at room temperature, facilitating injection and implantation, while rapidly solidifying at body temperature to form a stable structure. Differential Scanning Calorimetry (DSC) determined the lower critical solution temperature (LCST) to be approximately 31.3°C, confirming the material's thermosensitive characteristics. In a New Zealand white rabbit dry eye model, tear secretion in the experimental group significantly increased from 15.00 mm post-modeling to 20.75 mm after 7 days of treatment following thermosensitive lacrimal plug implantation ($p<0.01$). Histopathological analysis revealed a marked increase in conjunctival goblet cell count in the treatment group, further validating the therapeutic effect. The results indicate that this thermosensitive NIPAM lacrimal plug material demonstrates significant efficacy in improving tear secretion and promoting tissue repair. Its unique thermosensitive properties help address individual adaptability issues, while the drug-loading capability provides anti-inflammatory effects. This novel lacrimal plug presents an intriguing research direction for dry eye treatment, though further studies are needed to evaluate its safety and efficacy in clinical applications.

***Keywords*:** dry eye syndrome; thermosensitive lacrimal plug; N-isopropylacrylamide; anti-inflammatory; personalized treatment

1. Introduction

Dry eye syndrome is a multifactorial ocular surface disease characterized by tear film instability and ocular surface inflammation. In China, due to factors such as air quality issues and personal eye-use habits, dry eye syndrome shows a regional high prevalence [1]. Approximately 159 million individuals are affected, with 20% of moderate to severe cases requiring lacrimal plug treatment [2]. Existing lacrimal plug materials include silicone, Teflon, and poly(2-hydroxyethyl methacrylate), which function by occluding the lacrimal puncta to increase tear retention time, thereby alleviating dry eye symptoms [3][4]. However, these traditional lacrimal plugs present two major challenges in clinical application: firstly, standardized designs fail to accommodate individual anatomical variations, resulting in a high migration rate of up to 47%; secondly, the lack of in situ anti-inflammatory function leads to an inflammatory complication rate of approximately 5% [5].

To address these issues, this study proposes an innovative lacrimal plug design. We selected poly(N-isopropylacrylamide) (PNIPAM) as a thermosensitive hydrogel matrix, utilizing its unique thermosensitive properties to allow injection into the lacrimal duct at room temperature and rapid phase transition, providing occlusion functionality tailored to the lacrimal duct structure. Simultaneously, we loaded dexamethasone and mitomycin C into the PNIPAM gel, imparting anti-inflammatory and anti-fibrotic drug release capabilities to the lacrimal plug material.

This research aims to develop a lacrimal plug material with thermosensitive and anti-inflammatory properties, expecting to overcome the limitations of existing lacrimal plug materials, improve patient treatment efficacy and compliance, and provide a novel technical solution for the clinical treatment of dry eye syndrome.

Here's a professional English translation of the Materials and Methods section suitable for SCI journal submission:

2. Materials and Methods

2.1 Chemical Reagents and Materials

N-isopropylacrylamide (NIPAm), ammonium persulfate (APS), tetramethylethylenediamine (TEMED), and water-soluble type I collagen were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Dexamethasone (purity ≥98%) and

mitomycin C (purity ≥95%) were obtained from Shanghai Beyotime Biotechnology Co., Ltd. All chemical reagents were of analytical grade and used without further purification. Deionized water was prepared using a Millipore Milli-Q system.

2.2 Hydrogel Preparation

Following a previously reported protocol [6] with necessary modifications, type I collagen was first dissolved in deionized water (magnetic stirring for 120 minutes). Subsequently, NIPAm monomer was added to the solution and stirred for 60 minutes until completely dissolved. The initiator (APS) and accelerator (TEMED) were then added, and the reaction was completed under magnetic stirring at 25°C for 24 hours in a nitrogen atmosphere. The entire process was conducted under nitrogen. Finally, the hydrogel underwent dialysis treatment for 5 days using Cellu-sep membranes (Interchim, France) to remove residual NIPAm monomer and other unreacted chemicals, thereby eliminating potential biotoxicity.

2.3 Differential Scanning Calorimetry (DSC)

The lower critical solution temperature (LCST) of the hydrogel was measured using a differential scanning calorimeter (DSC 214 Polyma, NETZSCH, Germany). The analysis was performed under nitrogen flow (40 mL/min) over a temperature range of 20°C to 80°C, with a heating rate of 2°C/min. The LCST was determined by the endothermic peak of the DSC curve. Three replicate tests were conducted for each hydrogel sample group.

2.4 Rheological Testing

Rheological tests were performed using an AR-G2 rheometer (TA Instruments, Delaware, USA) equipped with a parallel plate geometry of 25 mm diameter and 0.5 mm gap. Temperature scans were conducted on each sample from 20°C to 40°C (heating rate of 0.5°C/s) at a frequency of 1 Hz and shear strain of 0.1% to monitor changes in complex viscosity (η*), storage modulus (G'), and loss modulus (G"). Three replicate tests were conducted for each hydrogel sample group.

2.5 Drug Loading

Dexamethasone and mitomycin C were loaded into the hydrogel using the soaking method. Lyophilized hydrogel samples were immersed in an aqueous solution containing dexamethasone (1 mg/mL) and mitomycin C (0.5 mg/mL) and left undisturbed at room temperature for 24 hours. Subsequently, the drug-loaded hydrogels were gently rinsed with deionized water to remove excess surface-adsorbed drugs, then freeze-dried at -80°C for 48 hours.

2.6 Establishment of Rabbit Dry Eye Model and Tear Secretion Detection

Healthy adult New Zealand white rabbits (weighing approximately 2.5-3 kg) were used as experimental animals, with a total of 4 rabbits (8 eyes). All rabbits were acclimatized for one week prior to the experiment, ensuring adequate water and standard feed. Animal experiments strictly adhered to ethical requirements for the protection and care of experimental animals and were approved by the relevant ethics committee. The dry eye model was induced by instilling 0.1% benzalkonium chloride eye drops into the right eye of the rabbits twice daily for 14 consecutive days. Benzalkonium chloride is a surfactant that disrupts tear film stability, causing ocular surface damage and inflammatory responses, gradually inducing dry eye symptoms. The left eye remained untreated as a control group. The right eye, serving as the experimental group, was treated with PNIPAM thermosensitive lacrimal plugs containing dexamethasone and mitomycin C after successful model establishment to evaluate their efficacy in treating dry eye disease. Tear secretion was measured using standard Schirmer test (SIT) strips placed in the lower eyelid for 5 minutes, after which the wetted length was measured (in mm). Tear secretion was assessed at the following time points: pre-modeling (Day 0), modeling Days 6, 12, and 15, and treatment Days 0 (day of lacrimal plug implantation), 3, and 7, recording tear secretion for both right (experimental) and left (control) eyes. All data were obtained through multiple repeated measurements.

2.7 Histopathological Analysis

Immediately after euthanasia, conjunctival tissue samples were collected from the rabbits. The right eye, with the implanted lacrimal plug, served as the experimental group, while the left eye served as the control. All tissue samples were immediately fixed in 10% neutral buffered formalin for 24 hours. Fixed tissues were dehydrated, cleared, embedded in paraffin, and sectioned into 4-μm thick slices for PAS staining. Stained tissue sections were observed under an optical microscope, focusing on the number and distribution of goblet cells on the conjunctival surface.

2.8 Data Analysis

Rheological performance and compression test data were plotted using Origin or MATLAB to generate curves of storage modulus and loss modulus against temperature, compressive strength against compression rate, and DSC curves to visualize results. Tear secretion detection results were presented as time curves to show trends before and after treatment.

Paired sample t-tests were used to analyze significant differences between the experimental and control groups at various time points for tear secretion detection results. Changes in the experimental group before and after treatment were assessed for statistical significance using t-tests. The significance level was set at 0.05, with p-values less than 0.05 considered statistically significant.

3. Results

3.1 Rheological Performance Test

Examining the relationship between temperature and hydrogel rheological properties, we determined that the phase transition temperature of the PNIPAM hydrogel was approximately 20.19°C. At this temperature point, the most significant change in rheological properties was observed, with a measured value of approximately -0.0909. Concurrently, the PNIPAM hydrogel exhibited distinct thermosensitive characteristics, with a lower critical solution temperature (LCST) of about 31.3°C, as determined by differential scanning calorimetry (DSC). Rheological tests revealed a significant inflection point around 30°C, where the hydrogel's flow properties began to decrease rapidly. Between 20°C and 30°C, flow properties remained relatively stable; above 30°C, particularly after 35°C, an accelerated decline trend was observed.

Further analysis demonstrated that the PNIPAm hydrogel exhibited excellent thermosensitivity and mechanical properties suitable for use as a lacrimal plug material in this study. Its storage modulus was 12,841 ± 8,803 Pa, ranging from 1.14 Pa to 22,967 Pa, indicating strong elasticity of the material at different temperatures, with a significant increase in storage modulus near human body temperature, providing sufficient mechanical strength to maintain the lacrimal duct occlusion effect. The loss modulus was 2,681 ± 1,686 Pa, ranging from 3.62 Pa to 4,247 Pa, reflecting the material's ability to absorb and dissipate energy, possessing appropriate viscosity to effectively reduce friction and stress concentration on lacrimal duct tissues, thereby enhancing patient comfort. The complex viscosity was 1,313 ± 895 Pa·s, ranging from 0.38 Pa·s to 2,332 Pa·s, demonstrating low viscosity and good fluidity at room temperature, facilitating easy injection into the lacrimal duct, while rapidly solidifying at body temperature to form a stable structure. This thermosensitive property of the material shows high application potential in lacrimal duct occlusion treatment, allowing injectability at room temperature and enhanced mechanical properties at body temperature, ensuring long-lasting and stable therapeutic effects (Figure 2).

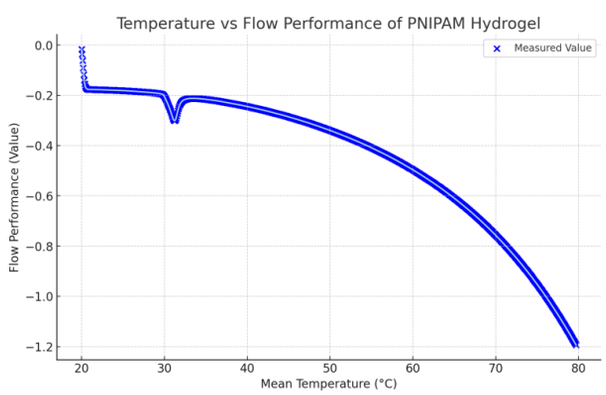

Figure 1: Temperature-Flow Property Relationship of PNIPAM Hydrogel

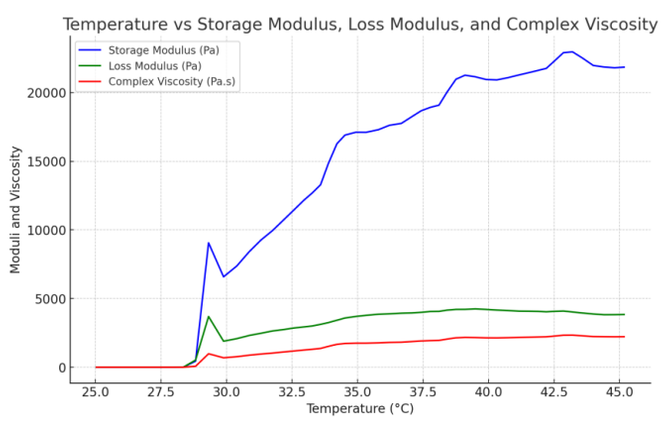

Figure 2: Effect of Temperature on Storage Modulus, Loss Modulus, and Complex Viscosity of PNIPAM Hydrogel

3.2 Evaluation of Rabbit Dry Eye Model

During the modeling phase, we observed dynamic changes in tear secretion. On the day of model induction (Day 0), there was no significant difference in tear secretion between the experimental and control groups, measuring 21.25 mm and 20.75 mm respectively ($t = 0.378$, $p = 0.731$), indicating comparable baseline conditions. By Day 6 of modeling, the experimental group showed significantly higher tear secretion (22.00 mm) compared to the control group (19.13 mm) ($t = 3.481$, $p = 0.040$), possibly due to a temporary increase in tear production caused by initial inflammatory responses. On Day 12 of modeling, tear secretion in both groups stabilized, measuring 18.00 mm and 17.75 mm respectively ($t = 0.174$, $p = 0.873$), showing no significant difference. This may indicate a gradual subsidence of inflammatory reactions and the onset of dry eye symptoms. Finally, on Day 15 of modeling, the experimental group exhibited significantly lower tear secretion (15.00 mm) compared to the control group (17.75 mm) ($t = -4.371$, $p = 0.022$), confirming the successful establishment of the dry eye model.

Upon entering the treatment phase, we observed that on the day of treatment initiation (Day 0), tear secretion in the experimental and control groups measured 16.50 mm and 16.75 mm respectively ($t = -0.132$, $p = 0.903$), showing no significant difference. This indicates that the lacrimal plug implantation did not produce an immediate significant effect. By Day 3 of treatment, tear secretion in the experimental group increased to 18.75 mm, while the control group measured 17.25 mm ($t = 1.567$, $p = 0.215$). Although there was an increase, it did not reach statistical significance, suggesting that the treatment effect had begun to manifest but had not yet fully developed. By Day 7 of treatment, tear secretion in the experimental group (20.75 mm) was significantly higher than in the control group (18.00 mm) ($t = 5.745$, $p = 0.010$), indicating that the thermosensitive lacrimal plug had produced a notable therapeutic effect at this time point (Table 1).

Table 1: Dynamic Changes in Tear Secretion in Rabbit Eyes During Dry Eye Model Establishment and Thermosensitive Lacrimal Plug Treatment

Time Point		Experimental Group	Control Group	t-value	p-value
Modeling Phase	Day 0	21.25 ± 0.50	20.75 ± 2.22	0.38	0.731
	Day 6	22.00 ± 2.58	19.13 ± 1.93	3.48	0.040*
	Day 12	18.00 ± 2.16	17.75 ± 0.96	0.17	0.873
	Day 15	15.00 ± 1.15	17.75 ± 0.96	-4.37	0.022*
Treatment Phase	Day 0	16.50 ± 3.79	16.75 ± 1.26	-0.13	0.903
	Day 3	18.75 ± 1.71	17.25 ± 0.50	1.57	0.215
	Day 7	20.75 ± 0.96	18.00 ± 0.00	5.74	0.010*

Note: Data are presented as mean ± standard deviation. Sample size n=4. * indicates $p < 0.05$, statistically significant.

Concurrently, we conducted a comparative analysis of tear secretion in the experimental group at key time points. From the day of model induction to Day 15 of modeling, tear secretion decreased from 21.25 mm to 15.00 mm, confirming the successful establishment of the dry eye model. From Day 15 of modeling to Day 7 of treatment, tear secretion significantly increased from 15.00 mm to 20.75 mm ($t = 5.745$, $p = 0.010$), indicating the substantial therapeutic effect of the thermosensitive lacrimal plug. Similarly, from Day 0 to Day 7 of treatment, tear secretion significantly increased from 16.50 mm to 20.75 mm ($t = 5.745$, $p = 0.010$), further corroborating the efficacy of the treatment (Table 2).

Table 2: Comparison of Tear Secretion in Experimental Group Rabbit Eyes at Key Time Points During Dry Eye Model Establishment and Thermosensitive Lacrimal Plug Treatment

Time Point Comparison	n	Before Treatment	After Treatment	t-value	p-value
Day 15 of Modeling vs Day 7 of Treatment	4	15.00 ± 1.15	20.75 ± 0.96	5.745	0.010**
Day 0 of Treatment vs Day 7 of Treatment	4	16.50 ± 3.79	20.75 ± 0.96	5.745	0.010**

Note: Data are presented as mean ± standard deviation. ** $p < 0.01$, indicating high statistical significance.

3.3 Histopathological Observations

In the modeling group, PAS staining revealed a significant reduction in the number of conjunctival goblet cells. This pathological change corresponded with the substantial decrease in tear secretion observed in the Schirmer's test (SIT), indicating that the ocular surface tissue had been affected by dry eye syndrome, resulting in suppressed tear secretion function. In the treatment group, following the implantation of the thermosensitive lacrimal plug, PAS staining demonstrated gradual tissue structure restoration. Compared to the modeling group, there was a significant increase in the number of conjunctival goblet cells on Day 7 of treatment, with the ocular surface tissue approaching normal conditions. This

observation aligned with the recovery of tear secretion, suggesting that the lacrimal plug not only effectively promoted an increase in tear secretion but also played a positive role in tissue repair. In the control group, PAS staining exhibited normal ocular surface tissue structure with a normal number of conjunctival goblet cells, indicating that the untreated ocular surface tissue maintained its normal state (Figure 3).

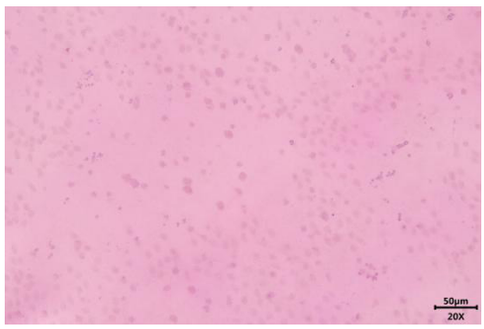

Figure 3A: Reduced number of conjunctival goblet cells on Day 15 of model establishment

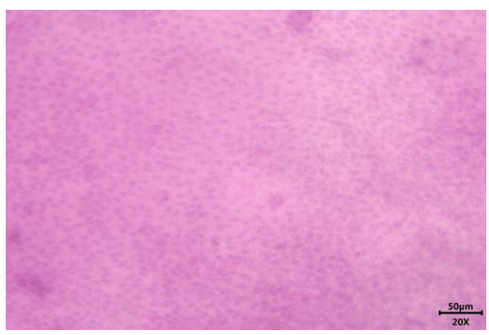

Figure 3B: Number of conjunctival goblet cells approaching normal levels on Day 7 of treatment

4. Discussion

In this study, we developed a thermosensitive lacrimal plug using N-isopropylacrylamide (NIPAM) hydrogel. This material remains hydrated below its phase transition temperature, allowing it to conform softly and closely to the anatomical structure of the lacrimal canaliculus. When the temperature rises to body temperature (37°C), the hydrogel dehydrates and becomes more rigid, effectively occluding the lacrimal duct and prolonging tear retention on the ocular surface. This thermosensitive characteristic shares similar advantages with the in situ-forming hydrogel lacrimal plug developed by Dai et al. [3], both capable of adapting to individualized lacrimal duct structures. Unlike traditional lacrimal plugs, the PNIPAM hydrogel incorporates dexamethasone and mitomycin C, conferring anti-inflammatory and anti-proliferative properties. This design can reduce inflammatory cell infiltration and inflammatory responses, thereby improving the long-term safety of lacrimal plug implantation. This drug delivery functionality aligns with the innovative development directions for lacrimal plugs discussed by Tost and Geerling [7].

Results from rheological performance and compression tests demonstrate that the hydrogel exhibits good elasticity and flexibility in the human physiological environment, facilitating stable implantation within the lacrimal duct. Under appropriate conditions, it displays high storage modulus and low loss modulus, indicating its ability to maintain tear retention. Brissette et al. [8] have shown that the retention rate of lacrimal plugs is a key factor in their clinical efficacy, and our thermosensitive hydrogel design holds promise for improving lacrimal plug retention rates.

During the establishment of the dry eye model, the experimental group showed a significant decrease in tear secretion, particularly on Day 15 of modeling, when tear secretion decreased to 15.00 mm, indicating exacerbation of dry eye symptoms and successful induction. This aligns with the phenomenon of reduced tear secretion in dry eye patients described in existing literature [9]. Following treatment with the thermosensitive lacrimal plug implantation, tear secretion in the experimental group gradually recovered, reaching a mean of 20.75 mm on Day 7 of treatment, approaching pre-modeling levels. This indicates that the thermosensitive lacrimal plug demonstrated good therapeutic efficacy in maintaining tear secretion. These results are similar to those reported by Burgess et al. [10] in their study of SmartPlug and silicone punctal plugs, both demonstrating the positive effects of lacrimal plugs on improving tear secretion.

The histopathological analysis, through PAS staining results, further confirmed the efficacy of this drug-loaded thermosensitive lacrimal plug. The lacrimal duct tissue in the modeling group showed a significant reduction in conjunctival goblet cells, consistent with the pathophysiological characteristics of dry eye syndrome. Conversely, the treatment group demonstrated significant tissue repair effects after implantation of the thermosensitive lacrimal plug, evidenced by a marked increase in conjunctival goblet cell numbers. This aligns with the recovery of tear secretion, indicating that the thermosensitive lacrimal plug played a positive role in promoting lacrimal duct tissue repair and improving the ocular surface microenvironment. This histological improvement is consistent with the treatment mechanism of lacrimal plugs summarized by Tost and Geerling [11]. Our study further extended the functionality of traditional lacrimal plugs by incorporating anti-inflammatory drugs. This innovative design not only physically retains tears but also directly improves ocular surface inflammation through drug action, echoing the concept of drug delivery systems for lacrimal plugs proposed by Best et al. [12], while our study further demonstrates the additional benefits of anti-inflammatory drug loading.

Compared to existing studies, the innovation of this research lies in emphasizing the individual adaptability and anti-inflammatory function of thermosensitive materials in dry eye treatment. Previous literature has reported the use of PNIPAM hydrogels in other biomedical fields, such as drug delivery systems and localized drug release systems [3]. These studies have demonstrated that PNIPAM hydrogels possess excellent biocompatibility and drug-loading capabilities suitable for controlled drug release. However, our study not only explored PNIPAM's drug-loading capacity but also focused on its unique advantages as a lacrimal plug material, including individualized adaptability and long-acting anti-inflammatory effects, making it more promising in dry eye treatment.

Furthermore, our research provides a potential material choice for the concept of 3D-printed personalized lacrimal plugs proposed by Khanna et al. [13]. Although our study did not directly involve 3D printing technology, the thermosensitive properties of PNIPAM hydrogels offer the possibility of manufacturing more precise, personalized lacrimal plugs in combination with 3D printing technology in the future.

However, this study has some limitations, such as a relatively small sample size and a relatively short observation period. Future research could consider increasing the sample size and extending the observation period to further validate the long-term efficacy and safety of the treatment. Additionally, exploring the impact of different dosages and release kinetics on treatment efficacy would be a valuable research direction. Overall, these results provide an experimental basis for novel treatment approaches to dry eye disease, paving the way for further research and applications.

5. Conclusion

In conclusion, the thermosensitive PNIPAM lacrimal plug material developed in this study has shown potential in preliminary animal experiments. This material provides new insights into addressing the limitations of existing lacrimal plugs in terms of adaptability to individual patient differences and anti-inflammatory functionality.

Acknowledgments

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