A Single-Center Clinical Study to Evaluate the Efficacy and Safety of Sutureless Ophthalmic Hydrogel for Corneal Wound Repair

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A Single-Center Clinical Study to Evaluate the Efficacy and Safety of Sutureless Ophthalmic Hydrogel for Corneal Wound Repair

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Protocol Summary

Study Title: A Single-Center Clinical Study to Evaluate the Efficacy and Safety of Sutureless Ophthalmic Hydrogel for Corneal Wound Repair.

Study Objective: To evaluate the safety and efficacy (therapeutic effect) of a sutureless ophthalmic hydrogel for repairing corneal defects in patients with corneal lamellar defects.

Study Subjects: Patients with corneal stromal defects caused by various etiologies, including those resulting from trauma or surgical lesion excision, without corneal perforation.

Study Design: Investigator-initiated, single-center, single-arm, open-label, single-dose trial.

Planned Number of Subjects:

A total of 30 cases

Study Period and Expected Participant Involvement Duration:

Study Period: April 2026 – April 2027

Expected Participant Involvement Duration:

The expected participation period for each subject in this clinical study is from the date of consent to the end of the observation period.

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1. Background Information

The cornea is a transparent tissue on the ocular surface that is vulnerable to trauma and infection. Corneal transplantation remains the primary treatment for corneal blindness. Due to a shortage of donor corneas and high surgical costs, 12.7 million patients worldwide are waiting for corneal donors, and only 1 in 70 patients receives corneal transplantation annually [1]. After corneal transplantation, the risk of graft failure due to immune rejection is lifelong. Therefore, addressing the shortage of donor corneas and developing new corneal grafts with good histocompatibility and low immunogenicity is highly desirable. A paradigm shift in corneal stromal repair is needed, analogous to the use of liquid sealants for filling tooth defects in dentistry. In corneal surgery, injectable hydrogels that require no sutures, adhere tightly to the corneal bed after implantation, and achieve anatomical repair are urgently needed

in clinical practice. With advances in ophthalmic biomaterials, various injectable corneal hydrogels have been developed. These materials have demonstrated excellent wound repair and pro-regenerative capabilities in animal studies.

Current clinical interventions for corneal injuries primarily include corneal suturing [2], corneal transplantation [3], conjunctival flap coverage [4], multilayer amniotic membrane transplantation [5], soft contact lens application [6], and injectable sealants [7]. Injectable sealants offer a rapid, sutureless repair method. In many ophthalmology centers, injectable corneal sealants are used for emergency management of corneal perforation caused by trauma or persistent ulcers.

Injectable corneal sealants mainly include cyanoacrylate glue, fibrin glue, and polyethylene glycol (PEG) gel. Cyanoacrylate glue offers the advantages of easy application and rapid sealing, and has been used for emergency closure of corneal perforations for over 50 years [8]. However, incomplete polymerization of cyanoacrylate glue leaves residual toxic monomers, and its degradation products release formaldehyde, alkyl cyanoacrylates, and other toxic compounds [9]. These toxic substances can irritate the cornea, leading to scarring and neovascularization, often necessitating subsequent corneal transplantation. Fibrin glue and PEG gel exhibit good histocompatibility and biodegradability in corneal repair [10, 11]. Nevertheless, these adhesives have drawbacks such as a narrow application window, poor adhesion, and the need for multi-component mixing, which limit their clinical use [12, 13]. Currently, there remains a lack of an injectable material that is convenient, exhibits good histocompatibility, and promotes corneal regeneration.

A hydrogel is a three-dimensional polymeric network with water-retaining capacity [14]. Hydrogel materials possess favorable mechanical properties,

cytocompatibility, and optical characteristics, making them suitable for the regeneration and repair of damaged corneas [15]. With advances in ophthalmic biomaterials, various types of hydrogels have been applied for corneal stromal repair. Based on clinical application type, corneal hydrogels can be categorized into preformed hydrogels and injectable hydrogels [16]. Preformed hydrogels are represented by collagen-based hydrogels [17]. Clinical studies have found that suture fixation of collagen-based hydrogels tends to cause graft degradation and stromal scar formation, limiting their further clinical application [18].

Injectable hydrogels are stimuli-responsive materials. Their liquid precursors, after injection into tissue defects, rapidly crosslink into solid gels under external stimuli or coupling agents. This minimally invasive delivery reduces patient discomfort, infection risk, recovery time, and costs, making them promising for wound repair [19]. Recent studies show that injectable corneal hydrogels overcome limitations of preformed hydrogels, offer good histocompatibility, enable sutureless repair of various stromal defects and perforations, and promote regeneration of epithelium, stroma, and nerves, potentially serving as an alternative to corneal transplantation [20].

Significant progress has been made in preclinical studies of injectable corneal hydrogels, demonstrating efficacy in repairing corneal ulcers and replacing conventional corneal transplantation in animal models; however, no reports on their clinical efficacy and safety are available.

2. Objectives

2.1 Primary Objective

The primary objective of this clinical study is to evaluate the safety of a sutureless ophthalmic hydrogel for repairing corneal lamellar defects. Safety will be assessed based on diagnostic and clinical examination findings, including the occurrence, severity, and frequency of all adverse

events (including systemic symptoms).

2.2 Secondary Objective

The secondary objective of this clinical study is to evaluate the efficacy (therapeutic effect) of the sutureless ophthalmic hydrogel for repairing corneal lamellar defects, using corneal thickness and corneal clarity as the primary outcome measures.

3. Study Design

3.1 Study Type

Investigator-initiated exploratory clinical study

3.2 Study Design

Investigator-initiated, single-center, single-arm, open-label, single-dose trial.

3.3 Evaluation Items

3.3.1 Primary Evaluation Items: Safety Evaluation Items

① Safety Evaluation Items (Systemic)

To evaluate the type, severity, and frequency of all related adverse events.

② Safety Evaluation Items (Ophthalmic)

- Intraocular pressure (IOP)
- Ocular surface inflammatory reaction
- Infection
- Corneal endothelial morphology and density

- Corneal epithelial healing time
- Hydrogel displacement or detachment

3.3.2 Secondary Evaluation Items: Efficacy Evaluation Items

- Corneal clarity / Corneal transparency
- Corneal thickness
- Visual acuity/Corrected visual acuity

3.4 Study Method Overview

This clinical study is a single-center, single-arm, open-label, single-dose trial evaluating the safety and efficacy (therapeutic effect) of a sutureless ophthalmic hydrogel for repairing corneal lamellar defects in patients with such defects.

The study consists of a screening period, an implantation period, and an observation period. After obtaining informed consent and completing registration, patients undergo screening examinations to confirm eligibility and absence of exclusion criteria. Surgery is performed under topical anesthesia. Following lesion debridement, the stromal bed is dried with a sponge, and a cotton pad is placed over the pupillary area to protect the fundus. The hydrogel, pre-liquefied in a 37°C water bath, is instilled into the lamellar defect using a sterile syringe, then irradiated with a 365 nm light source at an intensity of 18 mW/cm^2 for 30 seconds to achieve curing. A bandage contact lens may be applied after the procedure.

On postoperative day 1, gel integrity, thickness, and ocular surface inflammation are assessed using slit-lamp microscopy. During the 8-week observation period following gel injection, subjects are required to attend a total of four follow-up visits (Week 1, Week 2, Week 4, and Week 8). If any safety concerns arise from the trial results, necessary additional measures will be taken, and the specific ethics committee will be notified and must grant re-approval.

The safety of this clinical study will also be evaluated by the ethics committee. After completion of the first subject, the principal investigator may request an ethics

committee meeting based on the occurrence of serious adverse events or other relevant conditions, to seek advice on whether to continue the study or amend the clinical study protocol.

3.5 Expected Participant Involvement Duration

The expected participation period for each subject in this clinical study is from consent to the use of the sutureless ophthalmic hydrogel for corneal repair until the end of the observation period (8 weeks of postoperative follow-up).

4. Subject Inclusion/Exclusion/Discontinuation Criteria

4.1 Inclusion Criteria

Patients who meet all the following conditions are eligible for enrollment.

1）Patients with corneal stromal defects, including those caused by trauma or following lesion excision for infection, with a residual stromal thickness of ≥300 μm in the defect area.

2）Patients aged 18 to 85 years (inclusive, either sex) at the time of consent.

3）Patients who are capable of providing written informed consent voluntarily to participate in the study.

4.2 Exclusion Criteria

Patients meeting any of the following criteria will be excluded:

1）Patients with unexplained keratoconjunctival diseases.

2）Patients with severe dry eye, symblepharon, corneal neovascularization, or other ocular surface disorders.

3 ） Patients with systemic infectious diseases (positive for

bacteria/fungi/HBV/HCV/HIV/TP, etc.).

4）Patients with autoimmune diseases such as rheumatoid arthritis, Sjögren's syndrome, or graft-versus-host disease.

5）Patients with uncontrolled ocular diseases in the study eye.

6）Patients with major organ failure or other serious systemic conditions, including but not limited to: cardiac insufficiency; poorly controlled diabetes mellitus (fasting blood glucose >8 mmol/L despite glucose-lowering therapy); uncontrolled stage II or higher hypertension (blood pressure >160/100 mmHg despite antihypertensive therapy); history of malignancy within the past 5 years; severe immunodeficiency, etc.

6）Patients with psychiatric disorders that may interfere with treatment or evaluation.

8）Pregnant or lactating women, or women planning to become pregnant during the clinical study.

9）Patients deemed unsuitable for participation by the investigator.

10）Patients unable to complete follow-up visits.

4.3 Discontinuation Criteria

Subject participation will be discontinued under the following conditions, and after performing the scheduled examinations/observations/evaluations to the extent possible, the reason and course of discontinuation will be recorded in the case report form:

1) When the subject wishes to discontinue participation.

2) When, after obtaining consent, the subject is found not to meet the inclusion criteria or to violate the exclusion criteria.

3) When an adverse event occurs and it is determined that continuation of the study is difficult.

4) When the principal investigator and physician determine that the subject cannot comply with the protocol requirements.

5) When the clinical study itself is terminated.

Additionally, if a serious adverse event occurs, the principal investigator and physician shall report it to the ethics committee for regenerative medicine, etc.

5. Subject Informed Consent Acquisition/Registration

The principal investigator or physician provides oral and written explanation of the study's purpose, methods, etc., of the study to the subject and obtain written consent for study participation.

5.1 Informed Consent Acquisition

5.1.1 Preparation of "Informed Consent Form" and "Explanatory Document"

The "Informed Consent Form" and "Explanatory Document" are prepared by the principal investigator and approved by the ethics committee.

5.1.2 Timing and Methods of Informed Consent Acquisition

When obtaining consent from the patient, the principal investigator or attending physician will first provide the patient with the "Explanatory Document" and explain the study purpose/methods, etc., in an understandable manner. Next, they address the patient's questions about the study to confirm that they have fully understood the study content. Finally, the patient’s willingness to voluntarily participate in this clinical study based on free will will be confirmed, and this will be documented by the patient signing/sealing or initialing the dated "Informed Consent

Form".

When obtaining consent from the patient, the principal investigator or attending physician will confirm the patient’s contact information and the contact information of the patient’s family members or other emergency contacts, and record this in the medical records under the management of the participating institution.

5.1.3 Acquisition, Storage of "Informed Consent Form", and Case Registration

The principal investigator or attending physician who provided the explanation and the subject will each fill in the date, sign/seal or initial the "Informed Consent Form". The original copy will be stored by the principal investigator or the physician performing regenerative medicine, and a copy will be stored by the personal information manager after linkable anonymization. After obtaining consent, the principal investigator or attending physician will entrust the case registration center to register the case. In addition, before the subject participates in the study, a copy of the signed/sealed and dated informed consent form will be provided to the subject, and the date of delivery will be recorded.

5.1.4 Documentation of Informed Consent Acquisition Date

The principal investigator or the physician performing regenerative medicine will record the date of obtaining informed consent from the subject in the case report form.

6. Study Implementation Schedule

6.1 Pre-transplantation 1-7 days ~ Screening Period

6.1.1 Informed Consent Acquisition

The principal investigator or attending physician must obtain written informed consent from the patient and assign a subject identification code before performing any examinations/observations related to this clinical study. Informed consent must be obtained 1–2 days before surgery.

6.1.2 Eligibility Confirmation

Based on specified examinations/observations, the principal investigator and attending physician will confirm whether the candidate subject meets the inclusion criteria and does not violate the exclusion criteria (preliminary eligibility confirmation). Furthermore, based on the screening examination results, final eligibility confirmation will be performed within 1–2 days before surgery. Both preliminary and final eligibility confirmations will use the inclusion criteria in 4.1 and the exclusion criteria in 4.2.

6.1.3 Day Before Surgery (Day-1)

After admission, subjects will receive symptomatic treatment based on the underlying disease. Upon consent to surgery, relevant examinations will be completed on the day before surgery (Day –1), including:

1) Ophthalmic examinations: visual acuity (uncorrected and/or corrected), slit-lamp examination (anterior segment photography), intraocular

pressure measurement, corneal confocal microscopy, B-scan ultrasonography, and optical coherence tomography (OCT and/or panoramic OCT).

2) Concomitant medications.

6.1.4 Day of Surgery (Day 0)

1) On the morning of surgery, the subject's body temperature and blood pressure will be measured. If the subject's body temperature is ≥38° C or hypertension (systolic blood pressure >200 mmHg, diastolic blood pressure >120 mmHg) is present, the surgery will be discontinued or postponed.

2) The subject will undergo ophthalmic examinations: slit-lamp examination (anterior segment photography) to confirm that the ocular conditions meet the surgical indications.

6.1.5 Postoperative day 1

Subjects will undergo ophthalmic examinations, including visual acuity assessment, slit-lamp examination (anterior segment photography), and intraocular pressure measurement.

6.1.6 Discharge Date

1) Subjects will undergo ophthalmic examinations including uncorrected and corrected visual acuity, slit-lamp microscopy (anterior segment photography), intraocular pressure, corneal OCT/panoramic OCT, corneal topography, and biomechanical assessment.

2) The investigator will record the subject's ocular status (inflammatory reaction, corneal epithelial healing time, hydrogel adherence) and the presence or absence of adverse events, with detailed documentation in the

electronic medical record.

6.2 Observation Period: Visits 1～4 (Week 1～Week 8)

6.2.1 Visits 1 (Week 1), Visits 2 (Week 2), 3 (Week 4), 4 (Week 8)

Within each visit window, the following assessments and evaluations will be performed:

1) The investigator will inquire about the subject's symptoms and signs since the last visit and the occurrence of any adverse events, with detailed documentation in the electronic medical record.

2) Subjects will undergo ophthalmic examinations including: visual acuity (uncorrected and/or corrected), slit-lamp examination (anterior segment photography), intraocular pressure measurement, corneal OCT + panoramic OCT (weeks 1, 4, and 8), B-scan ultrasonography (week 2), confocal microscopy (weeks 4 and 8), corneal topography (weeks 1 and 4), biomechanical assessment (weeks 1 and 4), and fundus photography (week 4).

6.3 Unscheduled Visits

During each study period, if the subject experiences discomfort or signs/symptoms outside the scheduled examination time, an unscheduled visit may be arranged as needed to conduct corresponding examinations and record them in a timely manner.

7. Examination/Observation Items

7.1 Subject Background

Data to be collected: sex, date of birth, date of informed consent, target disease (diagnosis, surgical eye), present illness, etiological diagnosis, comorbidities, past medical history, presence of drug allergy,

and concomitant medications.

7.2 Subjective symptoms of the surgical eye

Examination method: Record subjective symptoms of the surgical eye (e.g., discomfort, redness, photophobia, tearing, foreign body sensation) through subject interview.

7.3 Basic Examination

7.3.1 Visual Acuity Examination

Method: Measure the 5-meter visual acuity (uncorrected and corrected) of the eye.

7.3.2 Intraocular Pressure Examination

Method: Measure the IOP of the transplanted eye using a handheld tonometer or non-contact tonometer.

7.3.3 Corneal Shape Analysis

Method: Anterior segment OCT of the surgical eye to evaluate hydrogel adhesion and filling, as well as the thickness and corneal clarity of the repaired area.

7.3.4 Corneal Topography Examination

Method: Corneal topography was performed to measure the curvature and morphology of the surgical eye.

7.3.5 Biomechanical Assessment

Method: Biomechanical assessment was performed to measure the strength and toughness of the surgical eye.

7.3.6 Confocal Microscopy Examination

Method: Confocal microscopy was used to assess corneal endothelial cell density and morphology, as well as stromal cell migration and regeneration and nerve regeneration in the hydrogel-filled area.

7.4 Ophthalmological Findings

7.4.1 Conjunctival Findings

Method: Slit-lamp microscopy was used to assess conjunctival hyperemia and discharge of the surgical eye.

7.4.2 Corneal Findings

Method: Slit-lamp microscopy was used to observe corneal epithelial healing time, corneal clarity (degree of corneal opacity), corneal stromal inflammation, and infection of the surgical eye. Corneal opacity was clinically graded from 0 to 4 (0 = completely transparent; 1 = slight opacity, not easily detectable under direct illumination; 2 = mild opacity, visible on slit-lamp examination; 3 = moderate opacity, partially obscuring the iris; 4 = severe opacity, concealing intraocular structural details).

7.5 Clinical Examination Items

7.5.1 Hematological Examinations

Laboratory examination items

Examination Item	Examination Indicators

Routine blood test	White Blood Cell count (WBC), Red Blood Cell count (RBC), Platelet count (PLT), neutrophil %, lymphocyte %, monocyte %, hemoglobin (HGB)
Urine routine	Urine occult blood, Urine pH, RBC count, WBC count, Urine glucose, Urine protein, Urine ketone bodies, Urine specific gravity
Blood biochemistry	Liver function: Total bilirubin (TBIL), Direct bilirubin (DBIL), Indirect bilirubin (IBIL), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Total protein (TP), Albumin (ALB); Renal function: Blood urea nitrogen (BUN), Creatinine (Cr); Electrolytes: Calcium (Ca^{2+}), chloride (Cl^{-}), potassium (K^{+}), sodium (Na^{+}), magnesium (Mg^{2+}); Myocardial enzyme profile: Creatine kinase (CK), CK-MB, Lactate dehydrogenase (LDH)
Blood glucose	Fasting blood glucose, Glycated hemoglobin (HbA1c)
Coagulation function (four items)	Activated Partial Thromboplastin Time (APTT), International Normalized Ratio

	(INR), Prothrombin activity (PT%), Prothrombin Time Ratio (PT-R), Fibrinogen (FIB), Prothrombin Time (PT), Thrombin Time (TT)
Viral screening (four items)	Hepatitis B core antibody (HBcAb), Hepatitis B e antigen (HBeAg), Hepatitis B surface antigen (HBsAg), Hepatitis B e antibody (HBeAb), Hepatitis B surface antibody (HBsAb), HIV (1+2) antibody (HIV-Ab), Hepatitis C antibody (HCV-Ab), Treponema pallidum antibody (TP-Ab)

7.5.2 Other Preoperative Ophthalmic Examinations

Items: Electrocardiogram (ECG), Serological infection screening (Syphilis, HBV, HCV). If the subject has severe ocular surface inflammation, conjunctival sac culture will be performed simultaneously.

7.6 Adverse Events

Investigate adverse events through examinations during hospitalization and outpatient visits.

8. Fabrication of Sutureless Ophthalmic Hydrogel

8.1 Basic Overview of the Material

Name: Sutureless Ophthalmic Hydrogel

Components: decellularized porcine corneal stroma, methacrylated gelatin

8.2 Volume Specification

Container	Volume (Filled Volume)
Syringe	500 μL

8.3 Fabrication of Sutureless Ophthalmic Hydrogel

8.3.1 Raw Material

Decellularized porcine corneal stroma, methacrylated gelatin (GelMA), recombinant human collagen.

8.3.2 Fabrication and Management of Sutureless Ophthalmic Hydrogel

8.3.2.1 Preparation of Decellularized Porcine Corneal Stroma Hydrogel (CECM)

1) Fresh porcine eyeballs were washed three times with sterile PBS in a laminar flow hood. After removing the epithelium with an epithelial scraper, central corneas were trephined using an 11 mm trephine. The endothelium was scraped off, and the corneas were soaked for 1 h in PBS containing penicillin–streptomycin.

2) Decellularization was performed by immersing the corneas in a solution containing 0.5% sodium lauroyl glutamate and 500 U/mL nuclease, with shaking at 120 rpm for 3 h at 37°C.

3) The corneas were washed six times with sterile PBS (15 min each), followed by two washes with sterile saline, and then freeze-dried.

4) To obtain the decellularized corneal stromal hydrogel (CECM), the samples were minced and soaked in 0.1 M HCl containing 10% pepsin for 18 h. The final concentration of the CECM solution was adjusted to 30 mg/mL, and it was stored at –20°C until use.

8.3.2.2 Preparation of Sutureless Ophthalmic Hydrogel

The sutureless ophthalmic hydrogel is formulated using methacrylated gelatin (GelMA) as the base matrix, which can be combined with biological materials such as decellularized porcine corneal stroma (CECM) and collagen. The specific protocol is as follows:

Scheme 1: CECM/GelMA composite system

The prepared 3% CECM solution was first adjusted to near-neutral pH with 1 M NaOH, then precisely calibrated to pH 7.4 with 500 mM Tris-HCl buffer (pH 7.4) to ensure a stable pH environment. The neutralized CECM solution, 10% GelMA, and 0.2% NAP were mixed in proportion and stirred with a magnetic stirrer for 30 min until a homogeneous and transparent mixture was obtained. The mixture was filtered through a sterile filter to remove potential impurities and microorganisms, then dispensed into 1 mL sterile syringes and stored at 4°C to prevent degradation.

Scheme 2: Decellularized porcine corneal stroma powder/GelMA composite system

The dried decellularized porcine corneal stroma powder was reconstituted with sterile saline to prepare a 3% powder solution, stirred thoroughly, and allowed to hydrate for 2 h to ensure complete dissolution. Then, 10% GelMA and 0.2% NAP were added to the neutralized powder

solution, and the mixture was stirred for 1 h to form a homogeneous composite precursor solution. After filtration through a sterile filter, the solution was dispensed into 1 mL sterile syringes and stored sealed at 4°C until use.

8.3.3 Sutureless Ophthalmic Hydrogel for Repairing Corneal Stromal Defects

On the day of surgery, whether a sutureless ophthalmic hydrogel can be used to repair the corneal stromal defect will be determined by the principal investigator and at least two conflict-free physicians, based on a comprehensive assessment of the subject's condition and the corneal stromal defect. Routine surgery will be performed, and the subject will first receive peribulbar block anesthesia. The surgical details (date of implantation, anesthesia method, presence of concomitant surgeries/procedures, operation time, diameter of hydrogel repair) and the presence of intraoperative complications will be recorded in the case report form.

8.4 Matters Concerning Ensuring the Safety of Trial Treatment

8.4.1 Expected benefits

Filling the stromal defect with the hydrogel is expected to restore corneal thickness, improve corneal clarity, and enhance visual acuity in the short term, while holding long-term promise for inhibiting corneal scar formation and promoting corneal stromal regeneration.

8.4.2 Expected harms

8.4.2.1 Hydrogel detachment or displacement

Judgment criteria: Hydrogel displacement or absence in the graft area observed on slit-lamp examination.

Management method: The principal investigator and physicians performing regenerative medicine will discuss the treatment plan during the observation period.

8.4.2.2 Infection

Judgment criteria: Presence of corneal infiltrate/ulcer, sharp increase of cells in the anterior chamber or hypopyon, ocular discharge, and conjunctival hyperemia.

Management method: The principal investigator and attending physician will immediately discuss the treatment plan. Surgical removal of the hydrogel and aggressive anti-infective therapy will be initiated. Corneal transplantation will be performed if necessary. Corneal smear and culture will be obtained to identify the causative pathogen.

9. Adverse Event Evaluation/Reporting

9.1 Definition of Adverse Events

An adverse event is any unfavorable or unintended sign, symptom, or disease that occurs in a subject, regardless of whether it is related to the sutureless hydrogel repair of corneal stromal wounds or other treatments.

Furthermore, in this clinical study, planned hospitalizations and examinations before study initiation, as well as hospitalizations specified in the clinical study protocol, are not considered adverse events.

Among adverse events, those meeting the following conditions are called serious adverse events:

Resulting in death (Fetal)

Life-threatening

Requiring therapeutic hospitalization or prolonging hospitalization

Resulting in persistent/significant disability/incapacity

Congenital anomaly/birth defect

Other serious events equivalent to the above

9.2 Adverse Events Evaluation

All adverse events observed from subject enrollment to the end of the study will be evaluated as described below. Clinically significant abnormal changes in laboratory values compared with preoperative levels will be reported as adverse events.

9.2.1 Onset Date of Adverse Events

Refers to the date when the adverse event is confirmed. However, for asymptomatic complications or incidental diseases, the date of diagnosis (the date on which the diagnostic examination was performed) is considered the onset date of the adverse event.

9.2.2 Expected Adverse Events

- Corneal inflammation
- Drug allergy caused by topical administration
- Delayed corneal epithelial healing
- Corneal epithelial implantation cyst

9.2.3 Severity Classification of Adverse Events

The principal investigator and attending physician will determine the severity of all adverse events occurring during this clinical study by replacing them with the severity of adverse events based on the following classification of side effects of drugs, etc.

Grade 1: Adverse events considered mild.

Grade 2: Non-serious adverse events but not mild.

Grade 3: Adverse events considered serious. That is, depending on the patient's constitution or condition at the time of occurrence, it may lead to death or permanent functional impairment that hinders daily life.

9.2.4 Adverse Events Outcome

The outcome of adverse events is classified as follows:

Recovery	Disappearance or resolution of symptoms/signs, normalization of test values, or recovery to pre-administration values.
Improvement	Reduction in the severity of symptoms/findings by at least 1 grade, near disappearance of mild symptoms/findings, or recovery of test values to near pre-administration values.

Unrecovered	No change in symptoms/findings or test values, worsening of the severity compared with the time of occurrence in the follow-up data on the last day of the observation period, irreversible congenital abnormalities, death in which the adverse event is not the direct cause and the adverse event remains unrecovered, recovery with sequelae, or partial recovery of symptoms/findings but partial confirmation as sequelae.
Death	A direct correlation is confirmed between death and the adverse event. Here, "confirmation of direct correlation" means that the adverse event is the cause of death or the adverse event significantly contributed to death. The outcome of an adverse event judged (presumed) to be an indirect cause of death in the same case is not considered "death".
Unknown	Cases where follow-up cannot be performed as described in the clinical study protocol after transplantation due to transfer, relocation, etc.

9.2.5 Outcome Date of Adverse Events

Record the date of recovery, improvement, unrecovery, or death during or after treatment. In addition, if the outcome date cannot be accurately specified, record the date of confirming the outcome content.

9.2.6 Correlation between adverse events and trial procedures and sutureless hydrogel repair of corneal wounds

The correlation with trial procedures and sutureless hydrogel repair of corneal wounds will be determined according to the following four categories.

Definitely related: Clear temporal correlation (including the course after surgery) and evidence of confirmed correlation;

Probably related: Clear temporal correlation (including the course after surgery), and factors other than the trial treatment, such as the primary disease, comorbidities, concomitant medications, and concurrent treatments, can be basically excluded;

Possibly related: Clear temporal correlation (including the course after surgery), other factors such as the primary disease, comorbidities, concomitant medications, and concurrent treatments can also be presumed, but the possibility caused by the trial treatment cannot be excluded;

Unrelated: No temporal correlation. It can be presumed to be caused by other factors such as the primary disease, comorbidities, concomitant medications, and concurrent treatments.

9.3 Management When Adverse Events Occur

During the subject's study participation, the principal investigator and attending physician will conduct necessary and appropriate examinations/observations and pay attention to the subject's safety. When an adverse event occurs, ensure that appropriate treatment is implemented,

pay attention to the subject's safety, and ensure that specialist diagnosis is obtained when necessary to strive to identify the cause.

In addition, during and after the subject's study participation, adequate medical measures will be taken for clinically significant serious adverse events related to the study.

After the end of the observation period, from the perspective of ensuring safety and scientific rationality, follow-up will be performed within the scope of insurance-based medical treatment if necessary. If it is known that a disease, disability, death, or infection suspected to be caused by the provision of this surgical trial has occurred, the principal investigator and attending physician shall promptly report the situation to the administrator of the medical institution performing regenerative medicine.

9.4 Adverse Events Recording

If a new adverse event occurs during the study period, record the following items in the adverse event column of the case report form.

Event name

Onset date

Severity

Outcome (recovery, improvement, unrecovered, death, unknown)

Outcome date

Correlation with trial procedures and transplantation of autologous

urine-derived epithelial cells

Management (discontinuation/continuance of the study, and treatment content for the adverse event)

9.5 Defects of Sutureless Ophthalmic Hydrogel for Corneal Defect Repair

Quality defects of the sutureless hydrogel refer to deviations in product quality that may occur after release determination and before delivery to the operating room or administration to the subject. Specifically, if any of the following defects are confirmed in the sutureless hydrogel, they will be recorded in the case report form:

1）Failure of the hydrogel to form a gel upon UV curing in vitro.

2）The hydrogel remains liquid at temperatures below 20°C.

3）The hydrogel fails to dissolve at 37°C.

4）White turbidity is observed in the hydrogel.

5）Damage to the packaging container is confirmed.

6）The product is not protected from light during transportation.

7）Other unexpected deviations.

The following items will be recorded in the case report form:

Implantation performed (1. No, 2. Yes)

Date of implantation

Date of defect occurrence

Defect classification (1. Contamination, 2. Defective product, 3. Performance, 4. Other)

Details of the defect

Presence of adverse events related to the defect (1. No, 2. Yes; if "Yes", record in the adverse events section)

9.6 Reporting of Adverse Events and Defects

When the following adverse events are known to occur during the implementation of this clinical study, the principal investigator and attending physician shall promptly report them to the administrator of the implementing medical institution.

10. Evaluation Items

10.1 Safety Evaluation Items

The primary safety evaluation item: [All adverse events including systemic symptoms].

The secondary safety evaluation items: [Adverse events caused by hydrogel filling] and [Adverse events caused by surgical procedures], which mainly include elevated intraocular pressure, ocular surface inflammatory reaction, hydrogel displacement or detachment, corneal infection, delayed corneal epithelial healing, and decreased corneal endothelial morphology and density, with the judgment criteria

determined by the investigator.

Primary Outcome Measures and Judgment Criteria:

Delayed corneal epithelial healing

Judgment criteria: Corneal epithelium remains unhealed for 7 consecutive days as specified in 7.4.2. If delayed healing is observed during follow-up, timely adjustment of medication is required.

Infection

Judgment criteria:

① Severe conjunctival hyperemia accompanied by ciliary hyperemia and purulent discharge as per 7.4.1 (Conjunctival findings);

② Corneal infiltration and decreased corneal clarity in the hydrogel-filled area as per 7.4.2 (Corneal findings).

Infection is diagnosed when either ①+② or ② alone is present.

Hydrogel detachment

Judgment criteria:

① Depression in the hydrogel-filled area as observed under 7.4.2 (Corneal findings);

② Decreased thickness of the hydrogel-filled area detected by corneal OCT (7.3.3);

③ Decreased corneal thickness in the hydrogel-filled area detected by corneal topography (7.3.4).

Hydrogel detachment is diagnosed when ① and/or ② and/or ③ is present.

Furthermore, considering the occurrence of side effects and abnormal changes in clinical laboratory values during this clinical study, the overall safety of each subject will be graded into the following four categories:

① **Safe:** No side effects or abnormal changes in clinical laboratory values during the study period.

② **Basically safe:** Mild study-related side effects occurred during the study period (relieved/resolved without special treatment), or clinically insignificant abnormal changes in clinical laboratory values occurred (resolved upon re-examination without

intervention).

③ **Safety concerns:** Moderate study-related side effects occurred during the study period (relieved/resolved only after symptomatic treatment), or clinically significant abnormal changes in clinical laboratory values occurred (requiring intervention/re-examination monitoring).

④ **Unsafe:** Severe study-related side effects occurred during the study period (requiring drug discontinuation/targeted treatment, or even endangering the subject's health), or marked abnormal changes in clinical laboratory values occurred (indicating organ dysfunction, requiring immediate intervention/drug discontinuation).

10.2 Efficacy Evaluation Items

Corneal Thickness

Judgment method: At each visit, based on the corneal thickness examination results described in 7.3.3, a corneal thickness improvement effect is considered present when the thickness reduction in the filled defect area does not exceed 20%.

Evaluation method:

① Effective: The improvement effect is observed at least once before Visit 4 and in a total of two or more visits including the final assessment.

② Ineffective: All cases other than ①.

Corneal Clarity (Degree of Corneal Opacity)

Judgment method: At each visit, based on the slit-lamp examination findings of the cornea described in 7.4.2, corneal clarity is considered good (stromal transparency after repair) when the corneal opacity score is ⩽2.

Evaluation method:

① Effective: Good corneal clarity is observed at least once before Visit 4 and in a total of two or more visits including the final assessment.

② Suggestive effective: Corneal thickness improvement effect is observed at least once before Visit 4, but corneal clarity is judged as poor at the final assessment.

③ Ineffective: All cases other than ① and ②.

Visual Acuity and/or Corrected Visual Acuity

Judgment method: At each visit, based on the visual acuity examination results described in 7.3.1, using distance decimal visual acuity, a visual acuity improvement effect is considered present when the visual acuity improves by at least one grade compared with the baseline (preoperative) assessment.

Evaluation method:

① Effective: The improvement effect is observed at least once before Visit 4 and in a total of two or more visits including the final assessment.

② Ineffective: All cases other than ①.

Judgment when "Insufficient Therapeutic Effect"

Judgment method: When at the final assessment, hydrogel degradation exceeds 20%, corneal opacity score is ≥3, and visual acuity is lower than baseline (preoperative visual acuity), the outcome is defined as "insufficient therapeutic effect."

11. Statistical Matters

11.1 Target Sample Size and Rationale

This clinical study aims to evaluate the use of a sutureless hydrogel for repairing corneal stromal defects. A minimum required sample size of 30 cases is set to confirm the reproducible safety trend of the hydrogel in corneal stromal repair, exploratorily assess its efficacy in repairing such defects, and provide foundational data for future clinical trials.

11.2 Demographic Characteristics

For demographic characteristics, the number of cases, mean, standard deviation, maximum, minimum, and median will be calculated for continuous variables. Other parameters required for descriptive reporting will be explored as needed.

11.3 Safety and Efficacy Analysis

For continuous evaluation items, the change from baseline and the summary statistics of the measured values will be calculated at each measurement time point. For discrete evaluation items, frequency summary at each time point or cross-tabulation between baseline and each time point will be performed. In addition, trend charts will be created if necessary.

11.4 Interim Analysis

No interim analysis will be performed.

11.5 Deviations from the Original Statistical Plan

If an analysis method different from that described in the statistical plan of this clinical study protocol is adopted, all changes will be recorded in the final report.

Regarding the handling of missing, unused, and abnormal data, the ethics monitoring committee will be consulted, and the handling method will be carefully determined on a case-by-case basis.

12. Deviations from the Study Protocol

12.1 Deviations Due to Emergency Situations

When the principal investigator and attending physician do not follow this clinical study protocol to avoid an emergency risk to the subject or for other medically unavoidable reasons, this fact must be recorded. The principal investigator will immediately submit a document describing the situation and its reasons to the medical institution administrator.

12.2 Deviations Due to Other Circumstances

If the principal investigator needs to modify this clinical study protocol, prior approval from the ethics committee must be obtained. When deviations occur due to other circumstances, the principal investigator will immediately submit a document describing the situation and its reasons to the medical institution administrator.

12.3 Compliance with Regulations, etc.

All personnel participating in this clinical study shall carefully read

and understand the content of the "World Medical Association Declaration of Helsinki", "Clinical Research Act", and laws related to the safety of "regenerative medicine", etc., which should be followed in all medical research involving humans, and comply with them.

12.4 Protection of Subject Privacy

Regarding the protection of subject privacy, the following matters will be observed:

After participating in this clinical study, the identification of subjects involved in the information obtained related to the study will be managed by linkable anonymization using the subject identification code assigned at the time of registration.

In the processing of examination/observation/evaluation results and the preparation/storage of case report forms, every effort will be made to protect the subject’s privacy.

12.5 Conflict of Interest and Research Funds

12.5.1 Conflict of Interest

The management of conflicts of interest of the principal investigator and attending physician will be based on the guidelines for conflict of interest management in regenerative medicine research followed by the principal investigator.

12.6 Periodic Reports

12.6.1 Report to the Specific Certified Regenerative Medicine

Committee

The administrator of the implementing medical institution will report the following matters to the ethics committee regarding the study status.

12.7 Compliance

This clinical study will be conducted in accordance with this study protocol and separately specified operating procedures. If compliance is not achieved, the situation will be recorded in detail.

The administrator of the implementing medical institution and the principal investigator will continuously confirm whether the study is being conducted properly in accordance with applicable rules, the regenerative medicine provision plan, and the study protocol, and at the same time take necessary measures to ensure proper implementation, such as study discontinuation, modification of the regenerative medicine provision plan or study protocol, if necessary.

If a person other than the principal investigator becomes aware of non-compliance, they shall report it to the principal investigator. If the principal investigator becomes aware of or receives a report, the process and measures will be recorded, and the administrator of the medical institution performing regenerative medicine will be reported based on the severity.

12.8 Handling and Record Storage of Data and Samples

Documents related to the implementation of this clinical study will

be stored in a personal information management hard disk by the document manager after being electronicized into PDF format, etc.

13. Research Costs and Compensation

We will provide you with the cell suspension free of charge. As a subject, if you suffer damage due to receiving the study drug treatment or following the study procedures correctly and in accordance with the researcher's guidance, the sponsor will compensate you for reasonable medical expenses incurred for the treatment of study-related injuries and reasonable compensation required by relevant Chinese laws and regulations.

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