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A first-in-human, prospective pilot trial of umbilical cord-derived mesenchymal stem cell eye drops therapy for patients with refractory non-Sjögren's and Sjögren's syndrome dry eye disease

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Abstract

Background Patients with refractory dry eye disease (DED) often face the threat of diminished visual quality and have limited responses to existing treatments. Ocular injection of Mesenchymal stem cells (MSCs) has recently emerged as a promising new therapeutic strategy for DED. Topical eye drops are the clinical favorable choice for drug administration in DED. To date, the clinical use of MSC eye drops has not been reported in settings. This clinical trial represents a groundbreaking exploration into the preliminary therapeutic potential and safety of umbilical cord MSC eye drops for patients with refractory DED, including both non-Sjögren's dry eye (NSDE) and Sjögren's syndrome dry eye (SSDE). The study also aimed to investigate the possible underlying mechanisms.

Methods In this open-label, prospective, single-arm, self-controlled trial, 11 NSDE and 5 SSDE patients received twice-daily MSC eye drops for two weeks, subsequent follow-up visits were scheduled at 4 weeks and 12 months after treatment. The primary efficacy was evaluated using the ocular surface disease index (OSDI) score, tear meniscus height (TMH), non-invasive break-up time (NIBUT), Schirmer I test (SIT), and corneal fluorescein staining (CFS) score. Secondary assessments focused on the evaluation of lipid layer, meibomian gland function, and bulbar conjunctival redness. Safety was monitored by recording adverse events (AEs) throughout the study. Changes in tear levels of interleukin-6 (IL-6), IL-17A, Mucin 5AC (MUC5AC), C–C chemokine ligand 20 (CCL20) and IL-23, along with proteomic alterations, were compared between baseline and T-week2.

Results Significant clinical improvements were observed in most symptoms and signs following MSC eye drops treatment in both NSDE and SSDE patients, particularly in tear production as measured by SIT and TMH, and the alleviation of meibomian gland blockage. The therapeutic effect on OSDI, NIBUT, and the lipid layer

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was more pronounced in NSDE patients compared to SSDE. No serious AEs were reported during the treatment and follow-up period. Post-treatment reductions in tear levels of IL-6 and IL-17A, along with an increase in MUC5AC, further confirmed the efficacy. Tear proteomic analysis indicated that the efficacy of MSC eye drops is associated with the inhibition of inflammation caused by T helper 17 (Th17) cells in both NSDE and SSDE groups.

Conclusions In this prospective exploratory clinical study, we have demonstrated that MSC eye drops might offer clinical efficacy and manageable safety in treating refractory DED for the first time, potentially bringing a new perspective on the treatment of such patients. Our research represents a preliminary exploratory endeavor, paving the way for future large-scale randomized positive-controlled trials.

Trial registration: ClinicalTrials.gov, NCT05784519. Registered 28 February 2023, https://clinicaltrials.gov/study/NCT05784519.

Keywords Mesenchymal stem cell eye drops, Non-Sjögren's dry eye, Sjögren's syndrome dry eye, Clinical efficacy, Safety, Tear proteomic analysis

Background

Dry eye disease (DED) is a complex chronic ocular condition characterized by tear hyperosmolarity and tear film instability, potentially resulting in ocular inflammation and damage. The increasing prevalence of DED poses a significant global health challenge [1, 2]. The primary causes of DED include reduced tear secretion (aqueous-deficient dry eye, ADDE), increased tear film evaporation (evaporative dry eye, EDE), and mechanisms involving both. Sjögren's syndrome (SS), an autoimmune disorder, is the main cause of ADDE, also known as Sjögren's syndrome dry eye (SSDE) [3]. In SS, lymphocyte infiltration into the lacrimal glands impairs the secretory function, leading to severe ocular discomfort [4]. SSDE, as a subtype of systemic disease-associated DED, often presents with more severe ocular symptoms compared to non-Sjögren's dry eye (NSDE). Clinical manifestations of DED, including ocular irritation, dryness, foreign body sensation, fatigue, and blurred vision [3], impose significant emotional and financial burdens on affected individuals [5, 6].

Current clinical treatment strategies for DED include pharmacological (such as artificial tears, topical corticosteroids and non-steroidal anti-inflammatory drugs) and nonpharmacological therapeutics (such as meibomian gland thermal pulsation and expression, intense pulsed light therapy) [7]. However, the efficacy varies significantly among individuals and caused sight-threatening side effects, the outcomes often falling short of satisfactory [8–10]. Recently, the emphasis in treatment has shifted towards restoring the natural ocular surface homeostasis. Despite these advancements, there are still several challenges and limitations in treatment, so there remain cases of refractory DED in clinical practice, where patients continue to endure severe ocular symptoms or signs despite undergoing one or more established therapy strategies. Patients often face the threat of diminished visual quality, which can significantly impair their daily activities and quality of life. This reality has sparked a growing interest in exploring alternative therapeutic approaches to help these patients navigate this challenging dilemma.

Emerging evidence highlights the importance of immune regulation as a crucial therapeutic target for both NSDE and SSDE patients [11, 12]. Mesenchymal stem (or stromal) cells (MSCs) have demonstrated potent anti-inflammatory and immunomodulatory capabilities, making them a focal point in therapeutic research for ocular disease inflammatory diseases [13]. Numerous studies have underscored the pivotal role of MSCs in animal models of ocular disease, with promising preclinical results paving the way for potential therapeutic applications in humans [14–16]. Additionally, MSCs have also shown favorable efficacy in autoimmune-associated dry eye [17, 18]. Recently, a research team in Denmark has been at the forefront of investigating the efficacy of allogeneic adipose-derived MSCs injected into the lacrimal glands for the treatment of DED. Their initial studies established the procedure for MSC therapy in patients with general DED and later expanded to include individuals with SS [19, 20]. The findings indicated that MSCs treatment was both significantly efficacious and safe for DED, as evidenced by enhanced tear secretion and reduced tear film osmolarity. Despite these advancements, current methods of MSCs administration on the ocular surface are primarily scaffold-based delivery and injection [15]. Common ocular injection methods include intravenous, subconjunctival, intrastromal, intracameral and lacrimal gland injections. Potential side effects associated with injection delivery include infection, pain at the injection site, periorbital edema, and in more severe cases, corneal opacity. Given that DED is an ocular disease, the direct application of topical eye drops may offer superior efficacy. Eye drops can make immediate contact with the cornea and conjunctiva, delivering a high concentration

of medication rapidly and effectively [21]. Additionally, eye drops are convenient and non-invasive. However, to date, there have been no published research on the clinical application of this approach. Therefore, it is hypothesized that eye drops could represent an ideal delivery method for treating refractory DED with MSCs, potentially simplifying treatment protocols and improving patient compliance.

Considering the ethical imperative to the initial human application of MSC eye drops, we have initiated this prospective, small-sample, single-arm trial. The study employed a within-subject pre- and post-treatment comparison design to assess the preliminary efficacy and safety of MSC eye drops in patients with refractory NSDE and SSDE. This trial is designed to generate valuable data on therapeutic outcomes and safety, these results will provide significant reference value for the conduct of randomized, positive controlled trials based on a larger patient population in the future.

Methods

Patient selection

This was an open-label, first-in-human, prospective, single-arm clinical trial conducted in the Department of Ophthalmology and Rheumatology at Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University. The trial adhered to the standards of the Declaration of Helsinki and the International Council for Harmonization Good Clinical Practice guidelines (ICH-GCP). Ethical approval was obtained from the local ethics committee (identifier, SC202200102), and the trial was registered on ClinicalTrials.gov (NCT05784519) and the Chinese Clinical Trial Registry (ChiCTR2200058115).

Participants included healthy individuals, as well as patients with NSDE and SSDE. The inclusion criteria for NSDE subjects are as follows: (1) Adults aged 18–70 years; (2) Symptoms and characteristics of dry eye consistent with the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) diagnostic criteria, including an Ocular Surface Disease Index (OSDI) score $\geq$ 13 or a 5-Item Dry Eye Questionnaire (DEQ-5) $\geq$ 6, with at least one positive indicator of homeostatic imbalance based on non-invasive break-up time (NIBUT), tear osmolarity and/or ocular surface staining [22]; (3) Lack of responses to one or more current therapy strategies for more than three months (The treatment history was detailed in Table S1). SSDE subjects had to meet all NSDE inclusion criteria and diagnosed with Sjogren's syndrome according to the ACR/EULAR criteria [23]. Exclusion criteria included recent eye surgery, infection, allergies within the past three months, or plans for pregnancy within the next 2 years, as well as any other conditions deemed exclusionary by the investigator. The study included 15 healthy subjects and 16 patients (11 NSDE and 5 SSDE), all of whom provided written informed consent. All patients underwent a one-week washout period during which they received no related treatment. This protocol was implemented to mitigate any potential residual effects of prior medications on the study's findings.

Clinical examinations

Clinical assessments included: OSDI questionnaire scores, tear meniscus height (TMH), NIBUT, lipid layer analysis, meibomian gland orifices and deletion, bulbar conjunctival redness, Schirmer I test (SIT) and corneal fluorescein stain (CFS) scores. Baseline tear samples were collected from both eyes of the patients for subsequent analysis.

The OCULUS Keratograph 5 M (Wetzlar, Germany) was used for examination, alongside standard SIT and CFS scoring as outlined in our previous research [24]. TMH and NIBUT recorded the measured values and scales respectively. NIBUT including both first (First NIBUT) and average tear film breakup time (Average NIBUT). Scales were documented for TMH, NIBUT, lipid layer, meibomian gland orifices and deletion, and bulbar conjunctival redness. 15 healthy subjects underwent the same clinical assessments as patients, with no follow-up treatment. Baseline characteristics and clinical features of all subjects are detailed in Table 1.

MSC eye drops treatment procedure

Umbilical cord-derived MSCs were sourced from the Nanjing Drum Tower Clinical Stem Cell Center. The production of clinic-grade MSCs adheres strictly to the Good Manufacturing Practice (GMP) level requirements according to our previous literature [25], ensuring the quality in the manufacturing process (Figure S1B-D). The detailed information on the production and transportation of MSC eye drops was shown in Figure S1A. Participants received bilateral ocular treatments twice daily for two weeks. The dosage was $5\times10\text{^}5$ cells per eye in a 50 µl volume. Sodium chloride eye drops (BAUSCH, China) served as the vehicle. MSC eye drops can be preserved over 90% cell viability for 48 h when stored at 4°(Figure S1E-F). Therefore, we provided each patient with a two-day supply of eye drops, containing $4\times10\text{^}6$ cells per 400 µl unit, for a total of seven shipments to complete the treatment process. To ensure precise dosage control, eye drop containers were sealed with micro-droppers (Tianyi, China) and refrigerated during transportation to patients, who were instructed to start treatment on the day of receipt and maintain storage at 4 °C. Tears were collected again at the end of the treatment period. A minimum washout period of 48 h

Table 1 Demographic and baseline characteristics in the healthy, NSDE and SSDE groups

	Normal	NSDE	*P* value	SSDE	*P* value
Number of patients	15	11		5	
Age (years)	41 ± 12.5	43 ± 15.9	0.831	49 ± 11.8	0.137
Gender, n (%)					
Female	9 (60.0)	9 (81.8)	0.024	4 (80.0)	0.157
Male	6 (40.0)	2 (18.2)		1 (20.0)	
Complication, n (%)					
None	30 (100)	20 (90.9)	0.092	8 (80.0)	**0.012**
Chronic bronchitis, recovered	0 (0)	2 (9.1)		0 (0)	
Gastroenteritis, flu, recovered	0 (0)	0 (0)		2 (20.0)	
OSDI score	3.9 ± 2.1	41.1 ± 22.1	**< 0.001**	48.8 ± 17.5	**< 0.001**
Number of eyes	30	22		10	
TMH (mm)	0.32 ± 0.06	0.16 ± 0.03	**< 0.001**	0.21 ± 0.07	**< 0.001**
TMH scale, n (%)					
Grade 1	17 (56.7)	0 (0)	**< 0.001**	0 (0)	**< 0.001**
Grade 2	13 (43.3)	1 (4.6)		3 (30.0)	
Grade 3	0 (0)	12 (54.5)		6 (60.0)	
Grade 4	0 (0)	9 (40.9)		1 (10.0)	
First NIBUT(s)	12.63 ± 3.57	5.43 ± 2.19	**< 0.001**	5.65 ± 4.56	**< 0.001**
Average NIBUT(s)	15.32 ± 3.02	7.66 ± 2.73	**< 0.001**	8.00 ± 4.63	**< 0.001**
NIBUT scale, n (%)					
Grade 1	16 (53.3)	0 (0)	**< 0.001**	2 (20.0)	**< 0.001**
Grade 2	13 (43.3)	3 (13.6)		0 (0)	
Grade 3	1 (3.4)	11 (50)		3 (30.0)	
Grade 4	0 (0)	8 (36.4)		5 (50.0)	
Lipid layer scale, n (%)					
Grade 1	6 (20.0)	0 (0)	**< 0.001**	0 (0)	**0.005**
Grade 2	21 (70.0)	4 (18.2)		4 (40.0)	
Grade 3	3 (10.0)	9 (40.9)		4 (40.0)	
Grade 4	0 (0)	9 (40.9)		2 (20.0)	
Meibomian gland orifices scale, n (%)					
Grade 1	13 (43.3)	0 (0)	**< 0.001**	0 (0)	**< 0.001**
Grade 2	16 (53.3)	7 (31.8)		4 (40.0)	
Grade 3	1 (3.4)	12 (54.6)		6 (60.0)	
Grade 4	0 (0)	3 (13.6)		0 (0)	
Meibomian gland deletion scale, n (%)					
Grade 1	20 (66.7)	2 (9.1)	**< 0.001**	0 (0)	**< 0.001**
Grade 2	10 (33.3)	8 (36.4)		6 (60.0)	
Grade 3	0 (0)	11 (50)		3 (30.0)	
Grade 4	0 (0)	1 (4.5)		1 (10.0)	
Redness scale, n (%)					
Grade 1	30 (100.0)	18 (81.8)	**0.015**	8 (80.0)	**0.012**
Grade 2	0 (0)	4 (18.2)		2 (20.0)	
Grade 3	0 (0)	0 (0)		0 (0)	
Grade 4	0 (0)	0 (0)		0 (0)	
SIT (mm/5 min)	18.4 ± 4.86	4.64 ± 3.4	**< 0.001**	2.5 ± 2.12	**< 0.001**
CFS score	0.3 ± 0.7	0.9 ± 1.6	0.052	3.1 ± 3.1	**< 0.001**

Both NSDE and SSDE patients had significant clinical features of DED compared with healthy subjects (Binocular outcomes were included in all subjects)

NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye, OSDI = Ocular Surface Disease Index, TMH = tear meniscus height, NIBUT = non-invasive break-up time, SIT = Schirmer I test, CFS = corneal fluorescein stain. The Data of age, OSDI, TMH, first/average NIBUT, SIT and CFS score are presented as the Mean ± SD. A p-value < 0.05 denoted statistical significance (highlighted in bold). The scale standards were shown in Table S2

was strictly implemented between the final application of MSC eye drops and tear fluid sample collection.

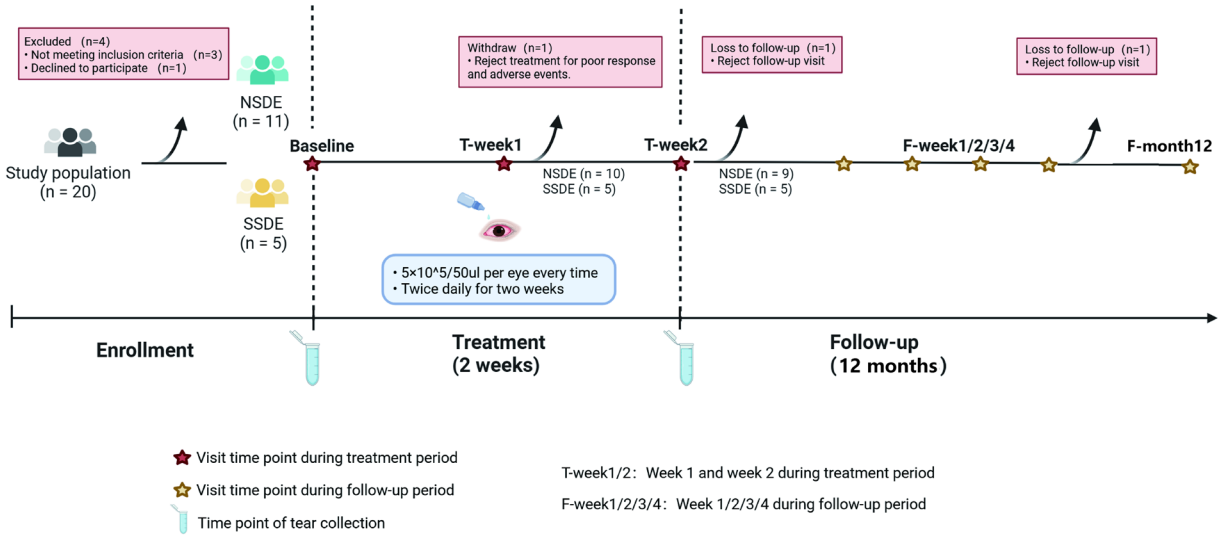
Outcome measures

The study included seven assessment time points: pre-treatment (baseline), post-treatment evaluations at 1 week (T-week1) and 2 weeks (T-week2) into the treatment phase. Subsequent follow-up visits were scheduled at 4 weeks (F-week1/2/3/4) and 12 months (F-month12) after treatment. (Fig. 1B).

Efficacy evaluation

1. Primary efficacy indicators: OSDI, SIT, CFS score, TMH and NIBUT.
2. Secondary efficacy indicators: lipid layer analysis, meibomian gland orifices and deletion, and bulbar conjunctival redness (Detailed scale standards are shown in Table S2).

A



B

		Baseline	Treatment		Sample size	Short-term follow-up				Sample size	Long-term follow-up	Sample size
		Visit	Visit1	Visit2	Complete all treatment visits	Visit1	Visit2	Visit3	Visit4	Complete all visits	Visit4	Complete all visits
		Week 0	T-week 1	T-week 2		F-week1	F-week2	F-week3	F-week4		F-month12	
OSDI score		✓	✓	✓	NSDE: 10 patients (20 eyes) SSDE: 5 patients (10 eyes)	✓	✓	✓	✓	NSDE: 9 patients (18 eyes) SSDE: 5 patients (10 eyes)	✓	NSDE: 8 patients (16 eyes) SSDE: 5 patients (10 eyes)
Assessment items of OCULU Keratograph 5M	TMH	✓	✓	✓					✓		✓	
	NIBUT	✓	✓	✓					✓		✓	
	Lipid layer scale	✓	✓	✓					✓		✓	
	Meibomian gland orifices scale	✓	✓	✓					✓		✓	
	Meibomian gland deletion scale	✓	✓	✓					✓		✓	
	Redness scale	✓	✓	✓					✓		✓	
SIT(mm/5min)		✓	✓	✓					✓		✓	
CFS score		✓	✓	✓					✓		✓	
Adherence assessment			✓	✓								
Safety assessment			✓	✓		✓	✓	✓	✓		✓	
Tear collection		✓		✓								

Fig. 1 The design of the clinical trial. **A** Flow diagram of the whole trial, **B** Overview of patient visits. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye, OSDI = Ocular Surface Disease Index, TMH = tear meniscus height, NIBUT = non-invasive break-up time, SIT = Schirmer I test, CFS = corneal fluorescein stain

Tear cytokines assay

Changes in tear levels of inflammatory cytokines IL-6 and IL-17A, along with the mucin MUC5AC, were measured as biomarkers for ocular surface inflammation and tear film integrity.

Medication compliance and safety

Medication compliance was assessed by recording the actual usage frequency. Safety was monitored through treatment-emergent adverse events (TEAEs), which were documented in a daily diary.

Enzyme linked immunosorbent assay (ELISA)

Tear samples collected at baseline and T-week2 were analyzed using ELISA. The methodology for tear collection followed previously established protocols [26]. The protein levels of IL-6, IL-17A, MUC5AC, CCL20 and IL-23 were quantified using respective ELISA kits per manufacturers' instructions. The final concentrations were calculated based on the dilution ratio.

4D-Data independent acquisition (4D-DIA) proteomics analysis

Tear samples from NSDE and SSDE patients were collected at baseline and T-week2. The protein extraction method involved eluting tear proteins from SIT strips using a solution containing PBS (Servicebio, China), 1% triton X-100 (BioFroxx, China), and 1% protease inhibitor (Solarbio, China). After overnight incubation at 4 °C and 6,000 g centrifugation at 4 °C for 10 min, the supernatant was obtained and stored at − 80 °C. For DIA proteomics quantification, total protein was measured using the BCA method. Proteins were reduced and alkylated using TCEP (tris (2-carboxyethyl) phosphine hydrochloride) and CAA (2-chloroacetamide), followed by overnight trypsin digestion (SignalChem). Digested peptides were desalted using a self-prepared SDB-RPS desalting column. Desalted peptides were loaded onto a timsTOF Pro mass spectrometer (Bruker Daltonics) coupled with an UltiMate 3000 RSLC nano-system (Thermo Fisher Scientific) and analyzed in diaPASEF mode. The raw DIA data were searched against the Human protein sequence database (2023–06-19, 20,423 entries) downloaded from Uniprot using DIA-NN software (V1.8.1). Proteins with a $|\log2\ FC| > 1$ (fold change > 2 or < 0.5) and $p < 0.05$ (paired t-test) were identified as differentially expressed proteins (DEPs). The heatmaps were conducted using the "ComplexHeatmap (2.16.0)" package. The Gene ontology (GO) classification and enrichment of DEPs were conducted using the "ggplot2 (3.5.1)"package (significant at $p < 0.05$). The protein–protein interactions (PPI) analysis network was analyzed for DEPs using the "igraph (1.5.1)" package. The version of R statistical programming is 4.2.1.

Statistical Analysis

Data analysis was performed using Prism (GraphPad 9.0). Comparisons between baseline and post-treatment data at T-week1/2, F-week1/2/3/4 and F-month 12 were made. Additionally, the differences in the variations of primary efficacy indicators between two subgroups of DED following treatment were compared. Continuous variables were first tested for normality using the Shapiro–Wilk test. For normally distributed data, repeated measures ANOVA followed by Dunnett's multiple comparisons test was conducted to identify significant differences between the baseline and each time point. For non-normally distributed data, the Friedman test was used to evaluate within-group effects over time, with Dunn's post-hoc test determining specific time points with significant changes. Adjusted p-values were reported to control for the Type I error due to multiple comparisons, ensuring the robustness of the findings.

Results

Twenty patients from the Department of Ophthalmology and Rheumatology at Drum Tower Hospital were preliminarily screened. Based on the inclusion and exclusion criteria, 16 patients (11 NSDE and 5 SSDE) provided written informed consent and participated in the study (Fig. 1A). The baseline data indicated that all patients exhibited significant DED characteristics compared to healthy controls, except for the CFS scores for NSDE patients, which did not reach statistical significance ($p = 0.052$) (Table 1). All 16 patients received MSC eye drops bilaterally, twice daily, at a dosage of 5×10^5 cells per 50 µl per eye, throughout a two-week treatment period, followed by a short-term four-week follow-up and a long-term follow-up at 12 months (Fig. 1A). The visit schedule and the examination items are depicted in Fig. 1B. The baseline visit was scheduled within one week prior to treatment initiation to minimize potential alterations in subject characteristics due to an extended recruitment period.

Changes in primary efficacy indicators from baseline

Primary efficacy outcomes included the OSDI score for subjective symptoms of ocular discomfort and the CFS score for ocular surface damage. Additional assessments for tear quality and quantity were conducted using the SIT, TMH and NIBUT.

The results of primary efficacy for the period of treatment and F-week1/2/3/4 were shown in Fig. 2.

For both NSDE and SSDE patients, the OSDI score decreased during MSC eye drops treatment, with significant reductions at T-week2 (NSDE: $p=0.0019$; SSDE: $p=0.0162$). To assess the durability of the treatment effects, evaluation continued at the end of each week throughout the follow-up period. NSDE patients showed a sustained significant decrease in OSDI scores, while SSDE patients exhibited a reduction that was not statistically significant (Fig. 2A).

The SIT results demonstrated a marked enhancement in tear secretion for both NSDE and SSDE patients. Significant improvements were observed in the NSDE group at T-week1 (6.11 mm, $p=0.0005$) and T-week2 (13.39 mm, $p=0.0001$), and in the SSDE group at T-week1 (4 mm, $p=0.0061$) and T-week2 (5.5 mm, $p=0.001$). These improvements were maintained at F-week4 (NSDE, $p=0.0006$; SSDE, $p=0.0048$) (Fig. 2B). Consistent therapeutic effects were also observed in TMH for both groups. At T-week1, NSDE patients showed a mean increase of 0.053 mm ($p=0.0012$), and SSDE patients showed a mean increase of 0.036 mm ($p=0.0346$). These increases were greater at T-week2, with NSDE at 0.123 mm ($p<0.0001$) and SSDE at 0.072 mm ($p=0.0004$), with effects persisting at F-week4 (NSDE, $p<0.0001$; SSDE, $p=0.0058$). The TMH scale change correlated well with the observed numerical changes (Fig. 2D-E). Representative images of tear meniscus in both groups pre- and post-treatment are displayed in Fig. 2F.

The NIBUT, a key measure of tear film quality, showed improvement in the NSDE group, with significant increases in both First NIBUT (T-week1: mean increase of 3.11 s, $p=0.0234$; T-week2: mean increase of 3.855 s, $p=0.0063$) and Average NIBUT (T-week1: mean increase of 3.66 s, $p=0.0139$; T-week2: mean increase of 4.42 s, $p=0.0001$) (Fig. 2G-H). However, in the SSDE group, the improvement in NIBUT was less pronounced, with only a slight increase in Average NIBUT at T-week2 ($p=0.0416$). The changes in the NIBUT scale further supported these findings (Fig. 2I). To further analyze the differences in treatment responses between these two subtypes of patients, we compared the variations of primary efficacy indicators between the two groups following MSC eye drops treatment. The results showed a noteworthy difference in the therapeutic response between the two subgroups, significantly influencing tear volume (SIT, $p=0.0077$) and TMH ($p=0.0325$) (Fig. 2J). Despite efforts, enrolling patients with high CFS score proved challenging, resulting in limited statistical difference despite a downward trend at T-week2 (NSDE, mean decrease of 1, $p=0.0356$; NSDE, mean decrease of 1.3, $p=0.1308$) (Fig. 2C).

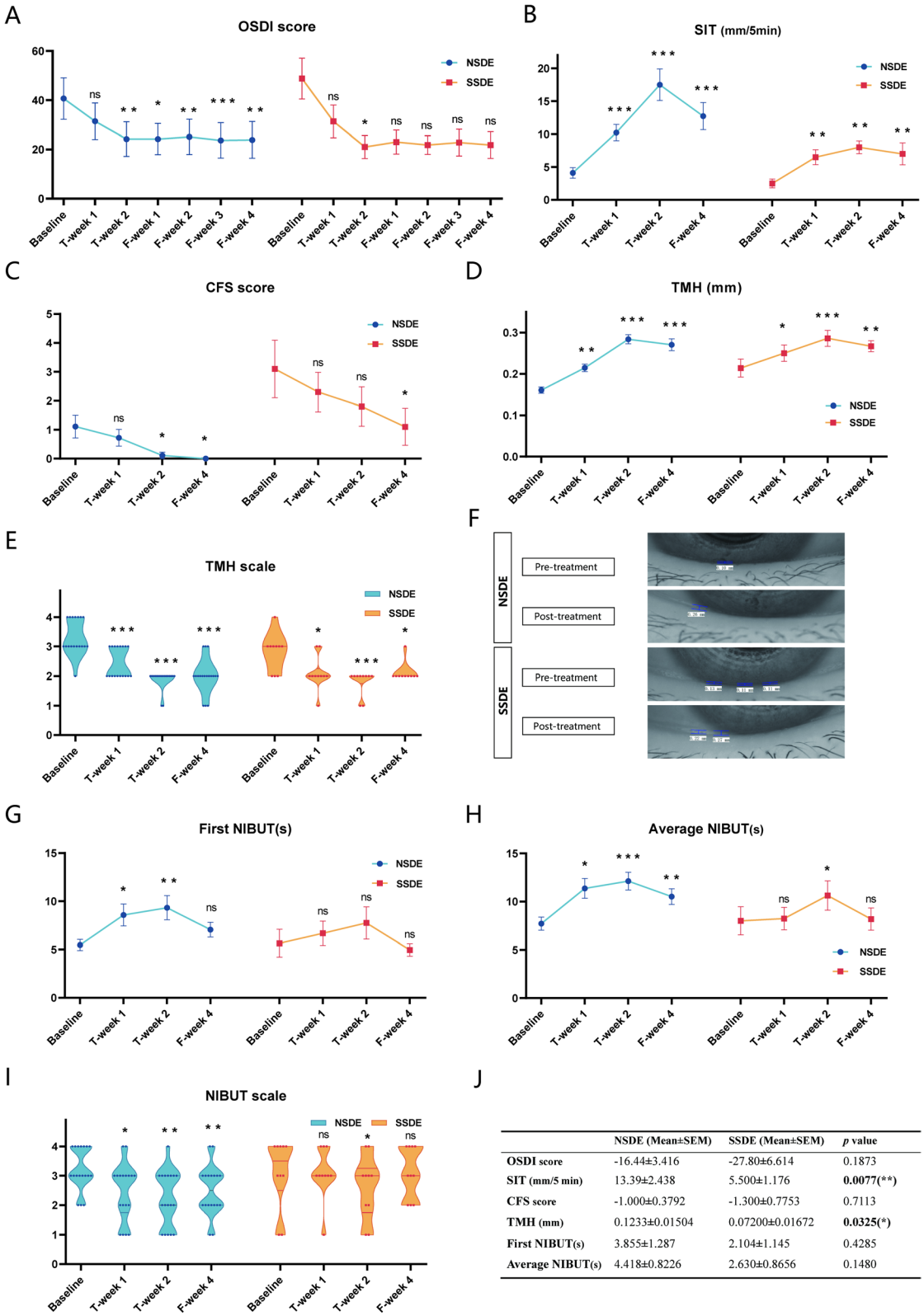
The measure outcomes of primary efficacy for F-month12 were presented in Table S3. We compared the examination results at F-month12 with both the baseline and the T-week2 outcomes. The results indicated that for patients with NSDE, there was still a suggestion of improvement in the primary efficacy indicators at F-month12 compared to baseline. This is evidenced by the significant improvements observed in OSDI ($p=0.0489$), TMH ($p=0.0003$), first NIBUT ($p=0.0149$), average NIBUT ($p=0.0002$), and SIT ($p=0.0038$). Additionally, when comparing the results of F-month-12 with the data from T-week2 for NSDE patients, aside from a slight decline in SIT ($p=0.0071$), there were no significant changes observed in the other primary indicators. The indicators for SSDE patients generally deteriorated at F-month 12 compared to T-week2, particularly in TMH ($p=0.0032$). These results indicated a promising long-term therapeutic effect of MSC eye drops for NSDE, while the response in SSDE patients appeared to be comparatively poor.

Changes in secondary efficacy indicators from baseline

The results of secondary efficacy for the period of treatment and short-term follow-up were shown in Fig. 3. Secondary efficacy outcomes concluded the assessment of meibomian glands and bulbar conjunctival redness. The meibomian glands secrete a lipid layer that overlays the ocular surface, serving a critical function in reducing tear film evaporation. The evaluation of meibomian gland health include assessments of lipid layer, gland orifices obstruction, and the glands deletion.

(See figure on next page.)

Fig. 2 OSDI, SIT, CFS score, TMH, and NIBUT at baseline, T-week 1, T-week 2 and follow-up period in the NSDE and SSDE groups. Clinical outcomes were measured in 9 patients with NSDE and 5 patients with SSDE who completed treatment and follow-up visits (Except for OSDI, all other items are assessed for both eyes. Data after MSC eye drops treatment from T-week1/2 and F-week1/2/3/4 were compared with baseline data (baseline). **A** OSDI score. **B** SIT value. **C** CFS score. **D** TMH value. **E** The scale of TMH. **F** Representative images of tear meniscus in the NSDE and SSDE groups pre- and post-treatment. **G** First NIBUT value. **H** Average NIBUT value. **I** The scale of Average NIBUT. **J** The differences in the variations of primary efficacy indicators between the two groups (NSDE, SSDE) following MSC eye drops treatment. Data are presented as the Mean ± SEM for **A**, **B**, **C**, **D**, **G**, **H** and **I**, while **E** and **I** are depicted on a scale from 1 to 4. The criteria for this scale are outlined in Table S2. ns$P>0.05$, *$P<0.05$, **$P<0.01$, ***$P<0.001$. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye, OSDI = Ocular Surface Disease Index, TMH = tear meniscus height, NIBUT = non-invasive break-up time, SIT = Schirmer I test, CFS = corneal fluorescein stain



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	NSDE (Mean±SEM)	SSDE (Mean±SEM)	*p* value
OSDI score	-16.44±3.416	-27.80±6.614	0.1873
SIT (mm/5 min)	13.39±2.438	5.500±1.176	**0.0077(**)**
CFS score	-1.000±0.3792	-1.300±0.7753	0.7113
TMH (mm)	0.1233±0.01504	0.07200±0.01672	**0.0325(*)**
First NIBUT(s)	3.855±1.287	2.104±1.145	0.4285
Average NIBUT(s)	4.418±0.8226	2.630±0.8656	0.1480

Fig. 2 (See legend on previous page.)

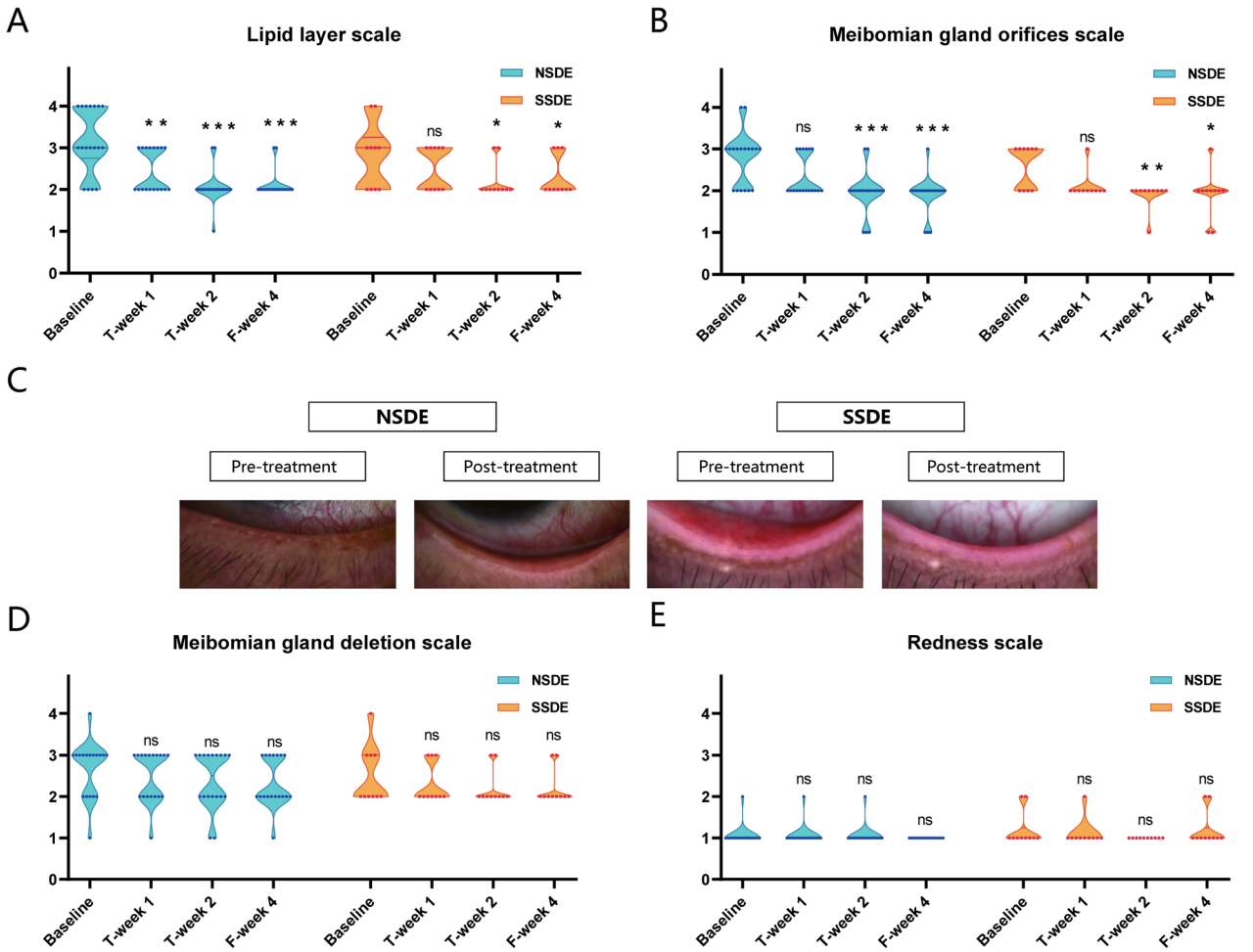

Fig. 3 Lipid layer, meibomian gland and redness scale at baseline, T-week 1, T-week 2 and F-week 4 in the NSDE and SSDE groups. Clinical outcomes were measured in 9 patients with NSDE and 5 patients with SSDE who completed treatment and follow-up visits (All items are assessed for both eyes). **A** The scale of lipid layer. **B** The scale of meibomian gland orifices. **C** Representative images of meibomian gland orifices in the NSDE and SSDE group pre- and post-treatment. **D** The scale of meibomian gland deletion. **E** The scale of bulbar conjunctival redness. Data are presented on a scale from 1 to 4, with the scale criteria detailed in Table S2. ns$P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye

Treatment with MSC eye drops significantly enhanced the lipid layer scale in NSDE patients, with improvements observed at T-week1 ($p = 0.0022$) and T-week2 ($p < 0.0001$), and sustained benefits evident at F-week4 ($p < 0.0001$). However, the therapeutic impact on the lipid layer in SSDE patients was less pronounced, showing statistical significance only at T-week2 ($p = 0.0119$) and F-week4 ($p = 0.028$), with a non-significant result at T-week1 ($p = 0.127$) (Fig. 3A). Notably, after a two-week treatment regimen with MSC eye drops, both NSDE and SSDE patients exhibited significant improvements in the obstruction of meibomian gland orifices, with $p = 0.0006$ for NSDE and 0.0069 for SSDE at T-week2. The therapy persistently alleviated the condition of meibomian gland orifice obstruction at F-week4, with $p = 0.0001$ for NSDE and 0.0287 for SSDE patients (Fig. 3B-C). However, no significant alterations were observed in the grading scales for meibomian gland deletion or bulbar conjunctival redness (Fig. 3D-E).

The measure outcomes of secondary efficacy for F-month12 were presented in Table S4. The secondary efficacy indicators for NSDE patients showed no significant changes. For SSDE patients, there was a notable decline in Meibomian gland function at F-month12 compared to T-week2, as evidenced by the changes of meibomian gland orifices ($p = 0.0378$) and meibomian gland deletion ($p = 0.0146$).

Medication adherence and safety assessment

We assessed medication adherence by calculating the percentage of actual medication frequency relative to the expected number. We found that 93.3% of patients demonstrated compliance exceeding 80% by the end of the treatment period (T-week2), affirming satisfactory adherence (Table 2).

Adverse events (AEs) were monitored throughout both the treatment and follow-up periods based on CTCAE5.0. While some patients experienced treatment-related adverse reactions, the majority were of grade 1 severity and had minimal impact on daily life. There were two instances of treatment discontinuation and one withdrawal due to AEs, with no serious adverse events reported within the treatment and short-term (4 weeks) follow-up period (Table 3). AEs that occurred in more than two patients during the treatment phase were documented, highlighting the most prevalent issues. The most frequently reported symptoms were indicative of ocular surface discomfort, including foreign body sensation and stinging eye pain (Table 4). These adverse reactions resolved within two days. Over the period of long-term (12 months) follow-up, none reported any AEs, validating the long-term safety of MSC eye drops.

Table 2 The medication adherence assessment at T-week1, T-week2 and summary

	Percentage of medication adherence			Summary
	100%	80–100%	< 80%	≥ 80%, total
T-week1, n[A] (%)	20 (66.7%)	10 (33.3%)	0 (0%)	30 (100%)
T-week2, n (%)	16 (53.3%)	12 (40.0%)	2 (6.7%)	28 (93.3%)

[A] n = number of eyes (The number of patients' eyes that completed treatment visits, 30 eyes in total)

Table 4 The medication safety assessment at T-week1, T-week 2 and follow-up period

Most common ocular AEs during treatment period[A]	Number of patients with this AE, n (%)
Ocular discomfort	4 (26.7%)
Excessive eye discharge	3 (20.0%)
Vision blurred	2 (13.3%)
Conjunctival hyperemia	2 (13.3%)

The most prevalent ocular AEs during the treatment period, along with the corresponding number of patients affected

[A] AEs suffered ≥ 2 patients

AEs = Adverse events

Changes in tear cytokines levels following treatment

Tears collected from NSDE and SSDE patients pre-and post-treatment were analyzed using ELISA. A total of 30 tear samples were assessed, comprising bilateral samples from 15 patients. Treatment with MSC eye drops resulted in a significant reduction in the levels of interleukin-6 (IL-6) and IL-17A in tears from all patients (Fig. 4A-B). Additionally, among the 30 tear samples, 20 collected from NSDE patients and 10 from SSDE patients, we observed that baseline Mucin 5AC (MUC5AC) levels in SSDE patients were significantly lower compared to those in NSDE patients (Fig. 4C). Subsequent analysis revealed an increase in MUC5AC levels in tears of SSDE patients following treatment (Fig. 4D).

Table 3 The medication safety assessment at T-week1, T-week2 and short follow-up period

	Treatment period (n = 16)				Follow-up period (n = 14)	
	T-week 1		T-week 2		F-week 1/2/3/4	
	Ocular[A] AEs	Non-ocular AEs	Ocular AEs	Non-ocular AEs	Ocular AEs	Non-ocular AEs
Patients with any AEs, n (%)	8 (50%)	1 (6.25%)	7 (43.75%)	0 (0%)	6 (42.9%)	0 (0%)
Grade 1	6 (37.5%)	1 (6.25%)	6 (37.5%)	0 (0%)	6 (42.9%)	0 (0%)
Grade 2	2 (12.5%)	0 (0%)	1 (6.25%)	0 (0%)	0 (0%)	0 (0%)
Treat-related AEs[B]	6 (37.5%)	0 (0%)	6 (37.5%)	0 (0%)	–	–
Serious AEs	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
AEs leading to interruption of intervention	1 (6.25%)	0 (0%)	1 (6.25%)	0 (0%)	0 (0%)	0 (0%)
AEs leading to discontinuation of study	0 (0%)	0 (0%)	1 (6.25%)	0 (0%)	0 (0%)	0 (0%)

The overview of ocular and non-ocular AEs

[A] The number of patients with ≥ 1 ocular AEs

[B] Considered by the investigator as suspected or related to study medication

AEs = Adverse events

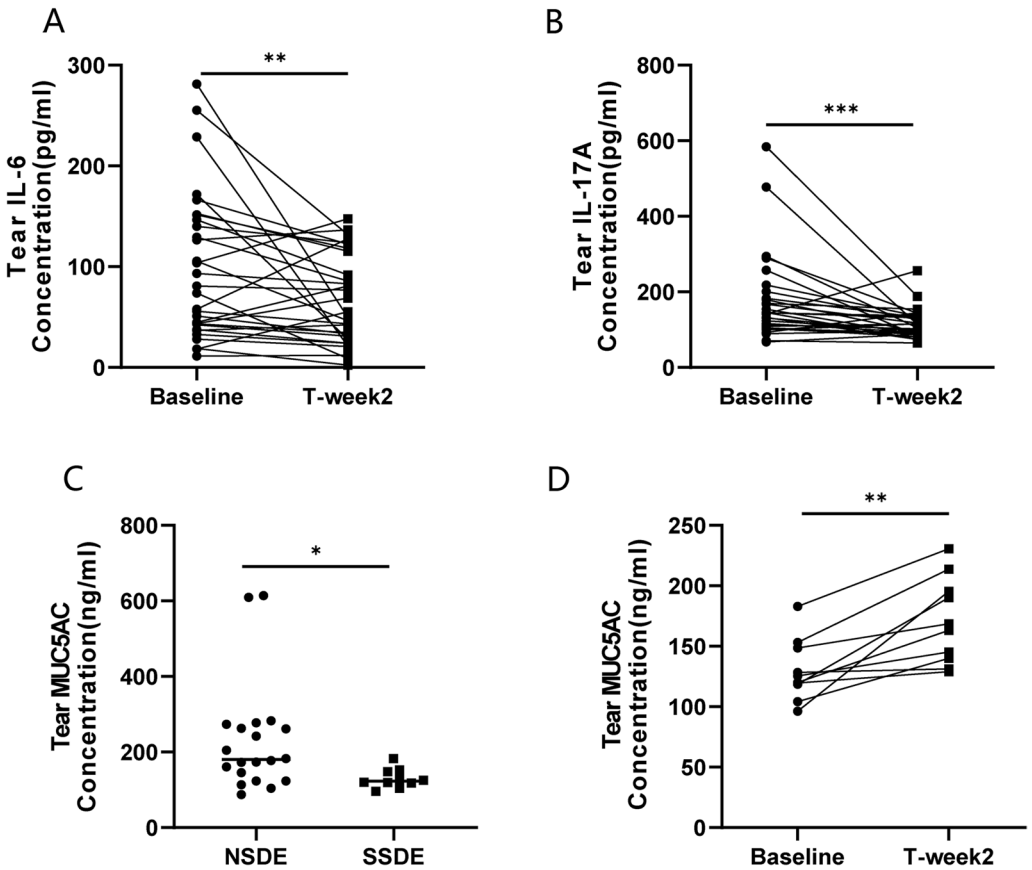

Fig. 4 Post-intervention versus changes of tear IL-6, IL-17A and MUC5AC levels from baseline. **A** The whole levels of IL-6 decreased in tears of all patients following treatment. **B** The whole levels of IL-17A decreased in tears of all patients following treatment. **C** Lower MUC5AC level in tears of SSDE than NSDE patients at baseline. **D** The levels of tear MUC5AC increased in tears of SSDE patients following treatment

Identification of differentially expressed proteins (DEPs) in tears from NSDE and SSDE patients Pre- and Post-treatment

To elucidate the mechanisms underlying the therapeutic effects of MSC eye drops in NSDE and SSDE patient groups, we conducted comparative proteomic analyses of tear samples collected pre- and post-treatment for both patient groups respectively. The comparative analysis was based on the changes observed post-treatment corresponding to pre-treatment conditions.

A total of 4,402 and 3,709 proteins were identified from tear samples of NSDE and SSDE patients, respectively. The PCA score plot reflected significant differences between post- and pre-treatment in both NSDE and SSDE patients respectively (Figure S2A). The volcano plot displayed that MSC eye drops treatment in NSDE patients was correlated with 53 DEPs, with 43 upregulated and 10 downregulated (Fig. 5A, fold change > 2, $p < 0.05$). In the SSDE group, treatment resulted in the identification of 60 DEPs, comprising 44 upregulated and 16 downregulated proteins (Fig. 5B, fold change > 2, $p < 0.05$). The heatmaps listed the upregulated and downregulated proteins in both patient groups (Fig. 5C-D).

Functional enrichment gene ontology (GO) analyses of DEPs in tears from NSDE and SSDE patients pre- and post-treatment

We conducted GO enrichment analyses for DEPs in both NSDE and SSDE groups across three levels: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). In cases where up- and down-regulated proteins were co-enriched for an item, we focused on the direction represented by the majority of enriched proteins. Parameters for the GO analysis are provided in Supplementary File 2.

At the BP level, The NSDE group showed greater enrichment in defense response, cellular component organization, endocytosis and immune relative process (Fig. 6A). The SSDE group exhibited an obvious enrichment in sensory perception, cell adhesion and differentiation, regulation of biological process and inflammatory immune response (Fig. 6B).

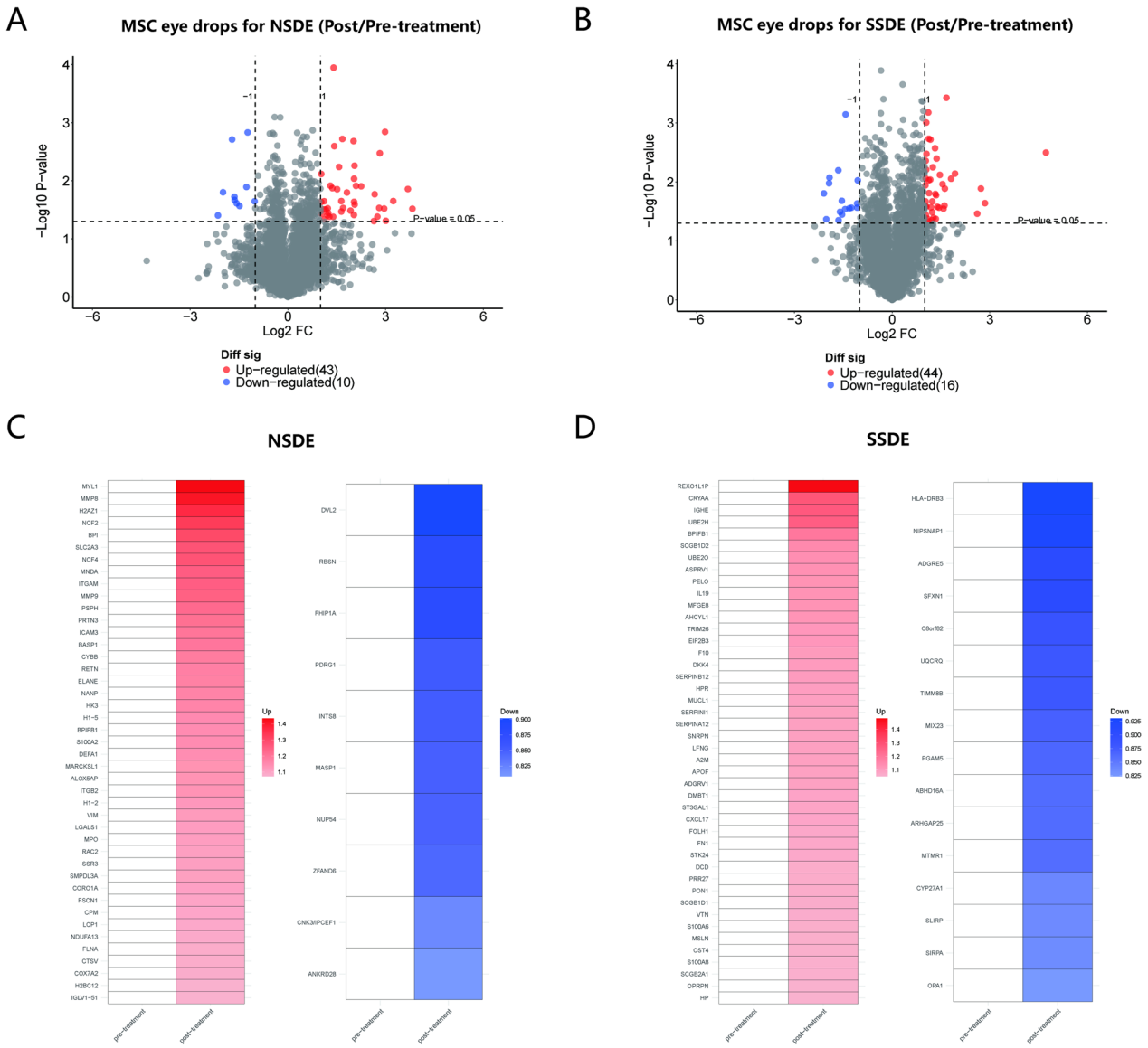

Fig. 5 Identification of DEPs in tears from the two patient groups post/pre-treatment respectively. **A**–**B** Volcano plots showed 53 DEPs in the NSDE group (**A**, n = 3) and 60 DEPs in SSDE group (**B**, n = 3) following treatment (Fold change > 2 and $p < 0.05$ were deemed statistically significant). (**C**–**D**) Heatmaps of upregulated and downregulated DEPs in NSDE group (**C**) and SSDE (**D**) group according to increasing and decreasing expression ratios. The detail expression ratios were shown in Supplementary File 1. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye, DEPs = differentially expressed proteins

To further elucidate the roles of these DEPs, we also conducted analyses at the levels of CC and MF. Our analysis revealed that the upregulated proteins in the NSDE group were predominantly involved in extracellular secretion, including vesicle and extracellular space (Figure S2B). The SSDE group showed upregulation of proteins involved in molecular function regulator activity (Figure S2C).

Protein–protein interactions (PPI) network analyses of DEPs in tears from NSDE and SSDE patients pre- and post-treatment

To elucidate the PPIs of DEPs in both NSDE and SSDE groups, we mapped the interaction networks in each group (Fig. 7). The DEPs were ranked based on their degree scores, which represent the number of

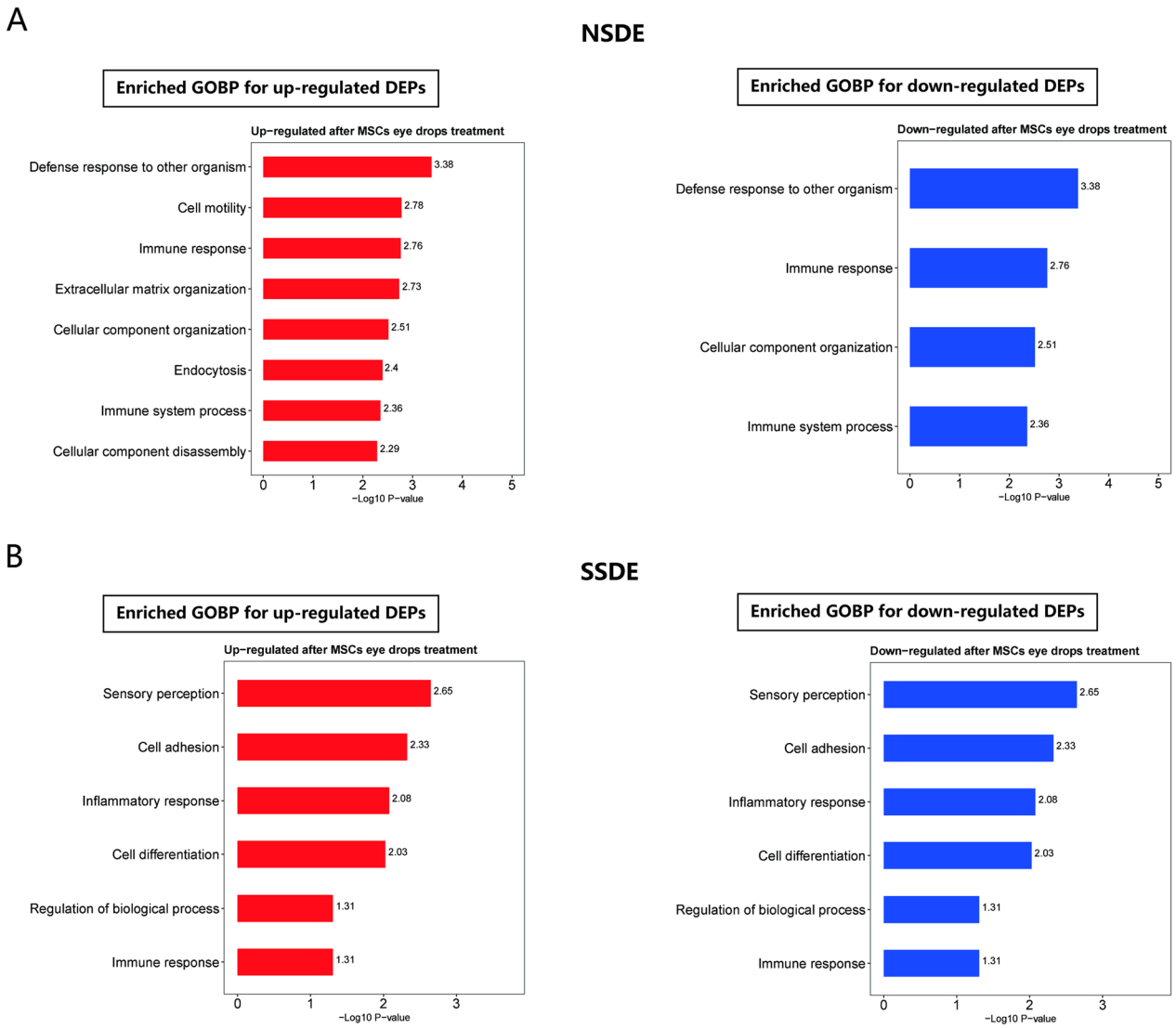

Fig. 6 GOBP analysis of DEPs in tears from the two patient groups post/pre-treatment respectively. Bar plots demonstrating significantly enriched GOBP ($p < 0.05$) for **A** NSDE group and (**B**) SSDE group. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye, GOBP = Gene ontology Biological Process

direct interactions (edges) each protein has with other network nodes (Supplementary File 3).

In the NSDE group, the hub proteins included immunomodulatory proteins, such as Matrix Metalloproteinase 8 (MMP8), MMP9, Proteinase 3 (PRTN3) (Fig. 7A). In the SSDE group, the core protein was inflammation-related protein Alpha-2-Macroglobulin (A2M) (Fig. 7B). These DEPs present potential targets for MSC eye drops therapy in treating patients with NSDE and SSDE.

Combined analysis of MSC eye drops-treated NSDE and SSDE groups

We performed an integrated analysis to elucidate the shared therapeutic mechanisms of MSC eye drops in both NSDE and SSDE cohorts, thereby informing future clinical applications.

An intersection analysis on the BP significantly enriched by individual GO analyses of both groups identified commonalities. As shown in Fig. 8A, the commonly enriched biological process might involve

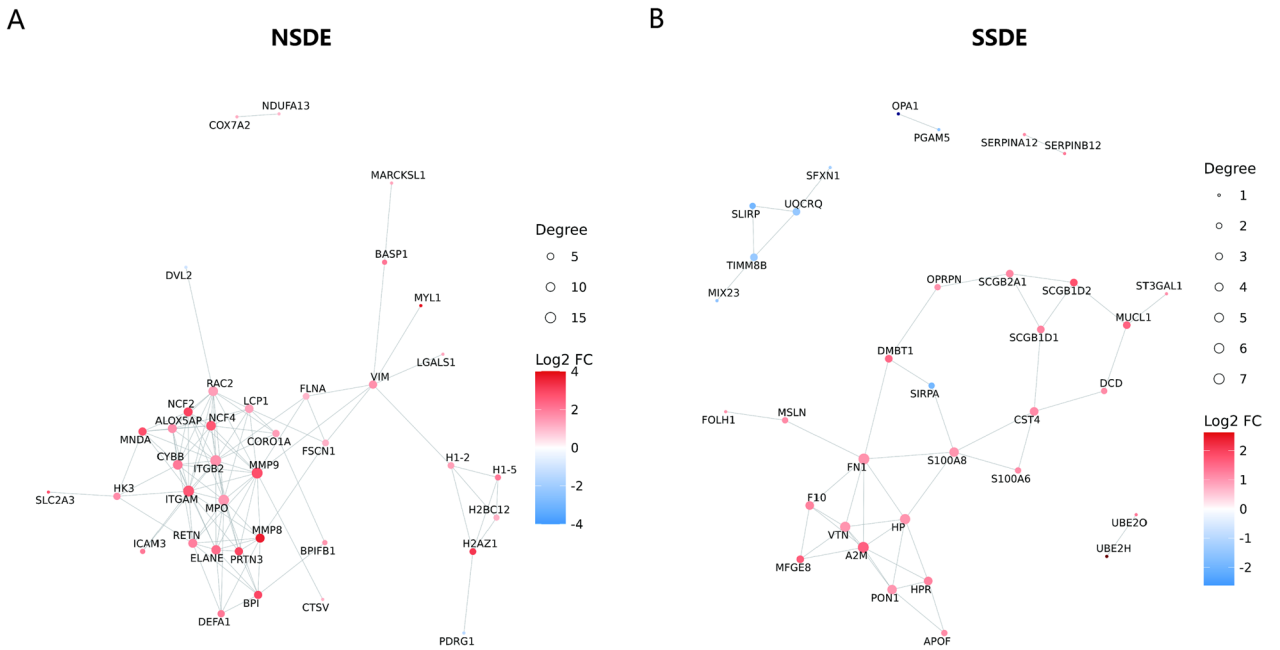

Fig. 7 PPI network analyses of DEPs in tears from the two patient groups post/pre-treatment respectively. PPI network for **A** NSDE and **B** SSDE group. Node colors represent the expression change of DEPs, with red for up-regulation and blue for down-regulation. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye, DEPs = Differentially expressed proteins

immune regulation. To further substantiate the immunomodulatory effects of MSC eye drops, we conducted assays for Th17-related cytokines CCL20 and IL-23. As depicted in Fig. 8B-C, a notable decrease in the levels of these cytokines post-treatment was observed, underscoring the potent anti-inflammatory capabilities of MSC eye drops.

Discussion

This study represents the first clinical application of umbilical cord-derived MSC eye drops for the treatment of refractory DED patients, including both NSDE and SSDE patients. This prospective study found that MSC eye drops demonstrated favorable efficacy in alleviating most clinical symptoms of both NSDE and SSDE patients, particularly in addressing tear deficiency and meibomian gland blockage. No serious adverse reactions were reported. Further assessment of tear cytokines IL-6, IL-17A, and MUC5AC corroborated the clinical findings. Additionally, tear proteomic analyses suggested that the regulation of immune responses might underlie the therapeutic effects of MSC eye drops.

Clinical results

The treatment of MSC eye drops appear well-suited for addressing tear deficiency, evidenced by significant enhancements in tear production and TMH in both patient subgroups (Fig. 2B, D-F). These results are consistent with prior research findings [27, 28]. Interestingly, the improvement effects of MSC eye drops on tear-related parameters (SIT, TMH) were less pronounced in SSDE patients compared to NSDE patients (Fig. 2J). Consistent with this finding, the long-term efficacy results also corroborate this trend (Table S3, S4). The variability in treatment outcomes can indeed be attributed to several reasons. Firstly, compared with NSDE, SSDE patients exhibit a more complex pathophysiology characterized by organic lacrimal gland damage, leading to tear deficiency [4]. Additionally, SSDE patients shown worse ocular signs, including a greater loss of meibomian glands compared to those with NSDE (Figure S3).The lipids secreted by the meibomian glands are crucial for stabilizing tear film distribution, and their loss exacerbates the tear film instability in SSDE patients [29]. Consequently, the poorer ocular condition in SSDE patients relative to NSDE limited their response to MSC eye drops treatment. Secondly, anti-inflammatory treatments for SSDE typically require several weeks to take effect, and the treatment period is usually longer than NSDE [30]. In our study, the two-week treatment period might be too short for SSDE patients. However, given the short-term effects observed, we believe that SSDE patients would benefit from a longer treatment duration. Additionally, the preservative-free formulation of MSC eye drops enhances patients' tolerability.

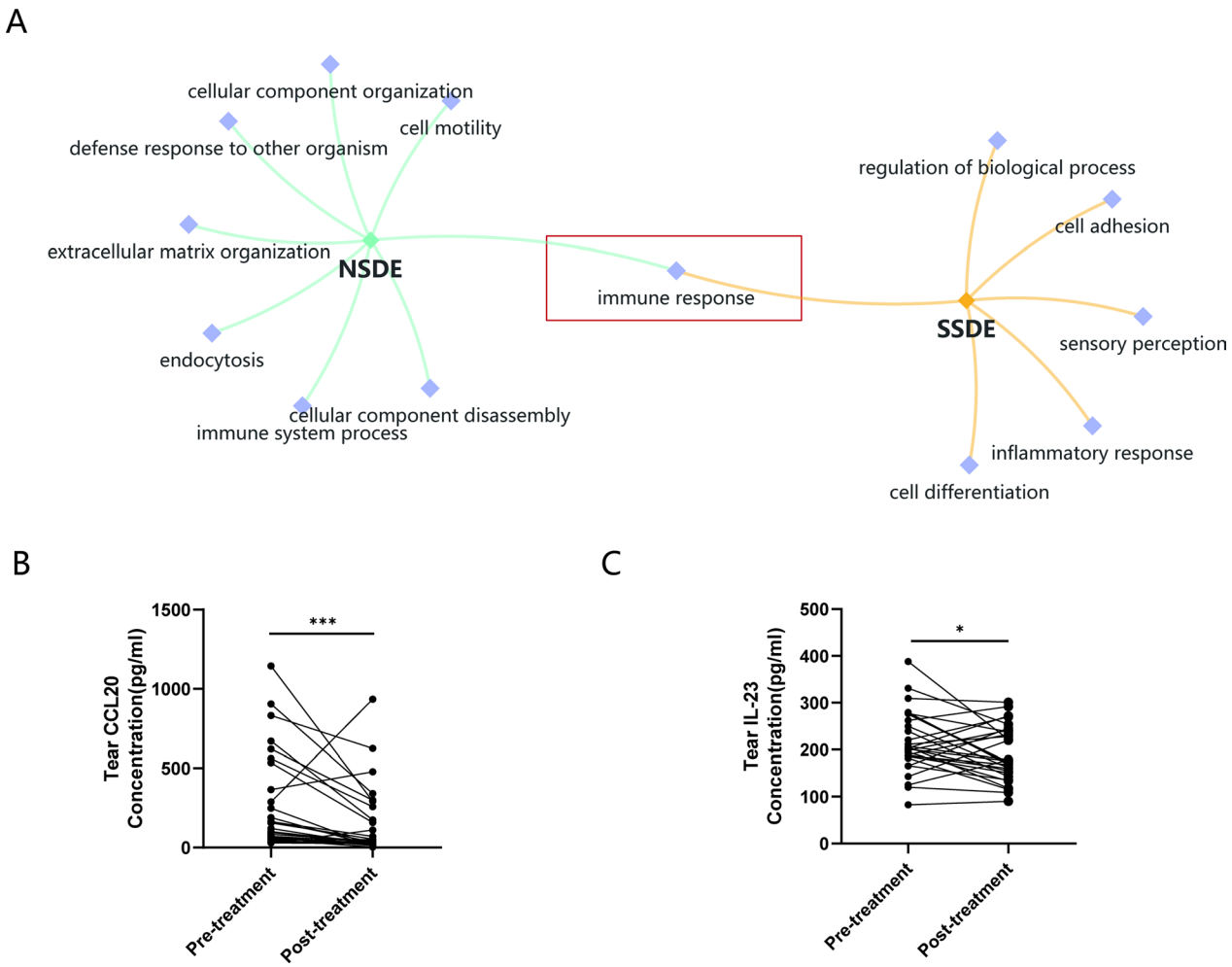

Fig. 8 Joint analysis of MSCs-treated NSDE and SSDE groups. **A** Network Venn diagram for GOBP terms of NSDE and SSDE groups. **B** The levels of CCL20 decreased in tears of all patients following treatment. **C** The levels of IL-23 decreased in tears of all patients following treatment. Abbreviations: NSDE = non-Sjögren's syndrome dry eye, SSDE = Sjögren's syndrome dry eye

For the secondary efficacy indicators, the results showed a significant improvement in meibomian gland blockage following treatment with MSC eye drops (Fig. 3B-C). However, the treatment had only a modest effect on the amelioration of meibomian gland deletion and bulbar conjunctival redness (Fig. 3D). Despite these, due to short follow-up period, we cannot conclude that MSCs have a weak capacity for ocular tissue repair.

Safety

In this study, patients received the treatment of MSC eye drops twice daily for two weeks, which was generally considered safe and well-tolerated. Only one case withdrawal from the study due to ocular discomfort associated with eye irritation, and no severe AEs were reported. The most frequently reported AEs during the treatment period were temporary ocular discomfort, including foreign body sensation and eye irritation. This might be attributed to the unstable tear film, corneal damage, and abnormal sensory neurology in patients, MSC eye drops have triggered a reflexive response on the unhealthy ocular surface [31, 32]. However, these adverse reactions were mild and short-lived. Long-term follow-up (12 months) with no adverse events reported indicates that the adverse events during the treatment period did not have a long-term impact on patients' lives.

Tear composition analysis

Changes in tear composition have become a central issue in the study of DED [33]. Inflammatory cytokines such as IL-6 and IL-17A in tears are recognized as the core components of ocular surface inflammation and are significant in the progression of DED [11], MUC5AC is an important mucin secreted by conjunctival goblet cells, playing a role of lubricating and wetting the ocular surface [34]. These markers have been widely used as

DED-related clinical detecting indicators [35, 36]. While our ELISA results substantiated the clinical findings, they offer only limited support for the underlying mechanism. In recent years, an increasing number of studies have applied tear proteomics technology to clinical research [37–39]. Therefore, we conducted tears 4D-DIA proteomic analysis to deeply explore the therapeutic mechanism of MSC eye drops and provide insights for future studies. To the best of our knowledge, this is the first study to use mass spectrometry to clarify the impact of MSC therapy on the entire tear proteome.

Impact of MSC eye drops on tear proteomics in NSDE and SSDE patient groups respectively.

Using 4D-DIA technology, we identified 4,402 proteins in tears from the NSDE group and 3,709 in the SSDE group, significantly improving the identification range compared with previous studies [37].

Among the DEPs in the NSDE group, MMP8, MMP9 and S100 Calcium Binding Protein A2 are recognized for their roles in maintaining ocular surface tissue integrity and modulating immune responses in the context of DED (Fig. 5C). MMP8, MMP9 and PRTN3 were the hub proteins in the PPI network of NSDE group (Fig. 7A). PRTN3, a serine protease implicated in inflammatory processes, is also located in the DED protein-interaction network [37] [40]. Additionally, novel findings included the significant upregulation of Myosin Light Chain 1 (MYL1). MYL1 has been identified as an important target for MSCs in maintaining bone tissue homeostasis [41]. Conversely, Mannose binding lectin-associated serine protease-1 (MASP1) markedly amplified inflammatory responses in myocardial ischemia/reperfusion injury [42]. The use of MSC eye drops resulted in downregulation of MASP1 in the NSDE group, indicating the anti-inflammatory effects..

Among the DEPs in the SSDE group, A2M was identified as the core protein in the PPI network, which exhibited an excellent anti-inflammatory effect (Fig. 7B) [43]. Upregulation of secretory mucins, such as mucin-like protein 1 (Fig. 5D), corresponded to the observed increase in MUC5AC (a key member of the mucin family) following MSC eye drops treatment (Fig. 4D). Additionally, upregulated Apolipoprotein F involved in lipid metabolism-related processes (Fig. 5D). Previous study has identified lipid transport-associated lipocalin-1 as a tear fluid marker for SS, underscoring the significant role of lipid metabolism regulation in the treatment of the SSDE patients [44].

For GO functional analysis, the results of GOBP analysis demonstrated the central role of immune regulation in MSC eye drops therapy (Fig. 6), consistent with previous proteomic studies on clinical tear samples [45, 46]. However, we found some difference between NSDE and SSDE groups.

In the NSDE group, the cellular components "extracellular space" and "vesicle" were significantly enriched, while the "vesicle" components were not enriched in the SSDE group (Figure S2B-C). The results shown that extracellular vesicle secretion might be essential in the effect of MSC eye drops on the NSDE patients. Exosomes are well-known crucial mediators of stem cell function [47]. Our previous research also demonstrated that exosomes derived from MSCs can ameliorate DED in mice by modulating the activity of dendritic cells [48]. Clinically, further investigation using corneal confocal microscopy is warranted to meticulously observe the changes in dendritic cells within the NSDE subjects' corneas.

Common mechanism of MSC eye drops treatment in NSDE and SSDE groups

By combining GOBP results from both groups, we found that MSC eye drops treat NSDE and SSDE through common pathways involving immune response (Fig. 8A). Research has demonstrated that T helper 1 and 17 (Th17) cells are pivotal in the chronic inflammation and autoimmune responses on the ocular surface, emphasizing a key link between Th17 cells and the pathology of DED, including its two subtypes [49, 50]. CCL20 has been proved that can enhance the migration of Th17 from the lymph node to the ocular surface [51]. IL-23 is linked with the activation of Th17 cells and is implicated in the proliferation of memory Th17 cells, which contribute to the perpetuation of chronic inflammation [52]. Activated Th17 cells predominantly secrete IL-17A, which is a critical factor in the perpetuation of inflammation on the ocular surface. Therefore, we have placed a significant emphasis on examining the effects of MSC eye drops on the Th17-relactived cytokines. We have observed a significant decrease in the levels of CCL20, IL-23 and IL-17A in tears following treatment with MSC eye drops (Fig. 4B, Fig. 8B-C). The results suggested that MSC eye drops may effectively modulate the immune response by targeting the CCL-20/IL-23-Th17-IL-17A axis, which is a critical pathway in the pathogenesis of DED. Our team is currently investigating the potential of MSCs to modulate Th17-related immune responses in an animal model. In summary, Th17-relatived immune regulation may be the shared mechanisms of MSC eye drops in the treatment of NSDE and SSDE.

Limitations

We acknowledge several limitations in our study. First, the small sample size and brief follow-up period may

limit the generalizability of our findings. Second, our study did not assess the well-documented tissue repair capabilities of MSCs. Thirdly, due to the constraints imposed by our study's small sample size, we were unable to explore the efficacy across a range of dosages or determine the optimal dosage for different patients. Fourthly, we acknowledge the potential incompleteness of our tear proteomics results due to sample insufficiency. Last, we did not validate the treatment for more nuanced dry eye subtypes, such as age-related meibomian gland dysfunction or graft-versus-host disease associated DED. In this study, the reported findings pertaining to safety and efficacy should be substantiated through future large-scale, randomized, controlled clinical trials.

Conclusions

Our study validated that the treatment of refractory NSDE and SSDE patients with MSC eye drops is a feasible and safe application method, particularly showing good and enduring efficacy for tear deficiency and meibomian gland blockage. This treatment might offer a convenient, efficient, and cost-effective therapeutic approach for refractory DED. The mechanisms may involve the suppression of Th17-related immune responses. Our study represents an initial foray into the realm of therapeutic efficacy and safety assessment. To further validate the therapeutic efficacy and explore the underlying mechanisms of treatment, it is necessary to conduct randomized controlled clinical trials with a larger sample size and an extended follow-up period.

Abbreviations

NSDE	Non-Sjögren's dry eye
SS	Sjögren's syndrome
SSDE	Sjögren's syndrome dry eye
OSDI	Ocular surface disease index
MSC	Mesenchymal stem cell
TMH	Tear meniscus heigh
NIBUT	Non-invasive break-up time
SIT	Schirmer I test
CFS	Corneal fluorescein staining
IL-6	Interleukin-6
MUC5AC	Mucin 5AC
TH17	T-helper 17
ADDE	Aqueous-deficient dry eye
EDE	Evaporative dry eye
DEQ-5	5-Item dry eye questionnaire
ELISA	Enzyme linked immunosorbent assay
TEAEs	Treatment emergent adverse events
AEs	Adverse events
4D-DIA	4D-data independent acquisition
DEPs	Differentially expressed proteins
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
PPI	Protein-protein interactions
BP	Biological process
CC	Cellular component
MF	Molecular function
MMP9	Matrix metalloproteinase 9
MYL1	Myosin light chain 1
MASP1	Mannose binding lectin-associated serine protease-1
PRTN3	Proteinase 3
A2M	Alpha-2-macroglobulin
CCL20	C–C chemokine ligand 20

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04292-8.

Additional file 1.

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Author contributions

Kai Hu proposed initial proposal. Di Zhang, Taige Chen, Qi Liang and Xuebing Feng designed the study and developed the protocol, so they shared the first authorship. Di Zhang and Taige Chen were responsible for study enrolment. Zeying Chen and Yun Tang completed the follow-up, recorded clinical and laboratory data. Di Zhang, Taige Chen, Qi Liang and Xuebing Feng contributed to the statistical analysis and analysis and interpretation of data. Bin Wang and Yiran Chu prepared UC-MSC eye drops. Di Zhang and Taige Chen drafted the manuscript. Kai Hu and Jiaxuan Jiang critically revised the manuscript. All authors revised the report and read and approved the final version before submission.

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Availability of data and materials

The authors declare that all the analysis data can be found in the main text and the Supplementary files. The raw data generated in this study are not publicly available due to the privacy protection policy of personal medical data at our institution but are available for non-commercial purposes from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the local ethics committee, all patients provided written informed consent before the procedure. The Ethical approval details are as follows: Title: Study on the curative effect of mesenchymal stem cell eye drops on dry eye disease. The institutional approval committee: Medical Ethics Committee of Drum Tower Hospital, Affiliated Hospital of Nanjing University Medical School. Approval number: SC202200102. Date of approval: 01 June, 2022. The human umbilical cord-derived MSCs utilized in this study were procured from the Clinical Stem Cell Center at Nanjing Drum Tower Hospital. As previously documented [25], ethical clearance was obtained from the Medical Ethics Committees of Nanjing Drum Tower Hospital for the project titled "Utilization of Clinical Patient Samples (Tissue/Blood/Body Fluids) and Aborted Fetal Tissue to Extract Stem Cells for Basic and Clinical Research in Regenerative Medicine and Treatment of Clinical Diseases" (Approval No. 2017–161-08; Approval Date: November 30, 2017). Informed consent forms were signed by all donors prior to sample collection, ensuring adherence to ethical guidelines for research involving human subjects.

Consent for publication

The patients gave consent for the publication of the data.

Competing interests

The authors have declared that no conflict of interest exists.

Artificial intelligence
The authors declare that they have not use AI-generated work in this manuscript.

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