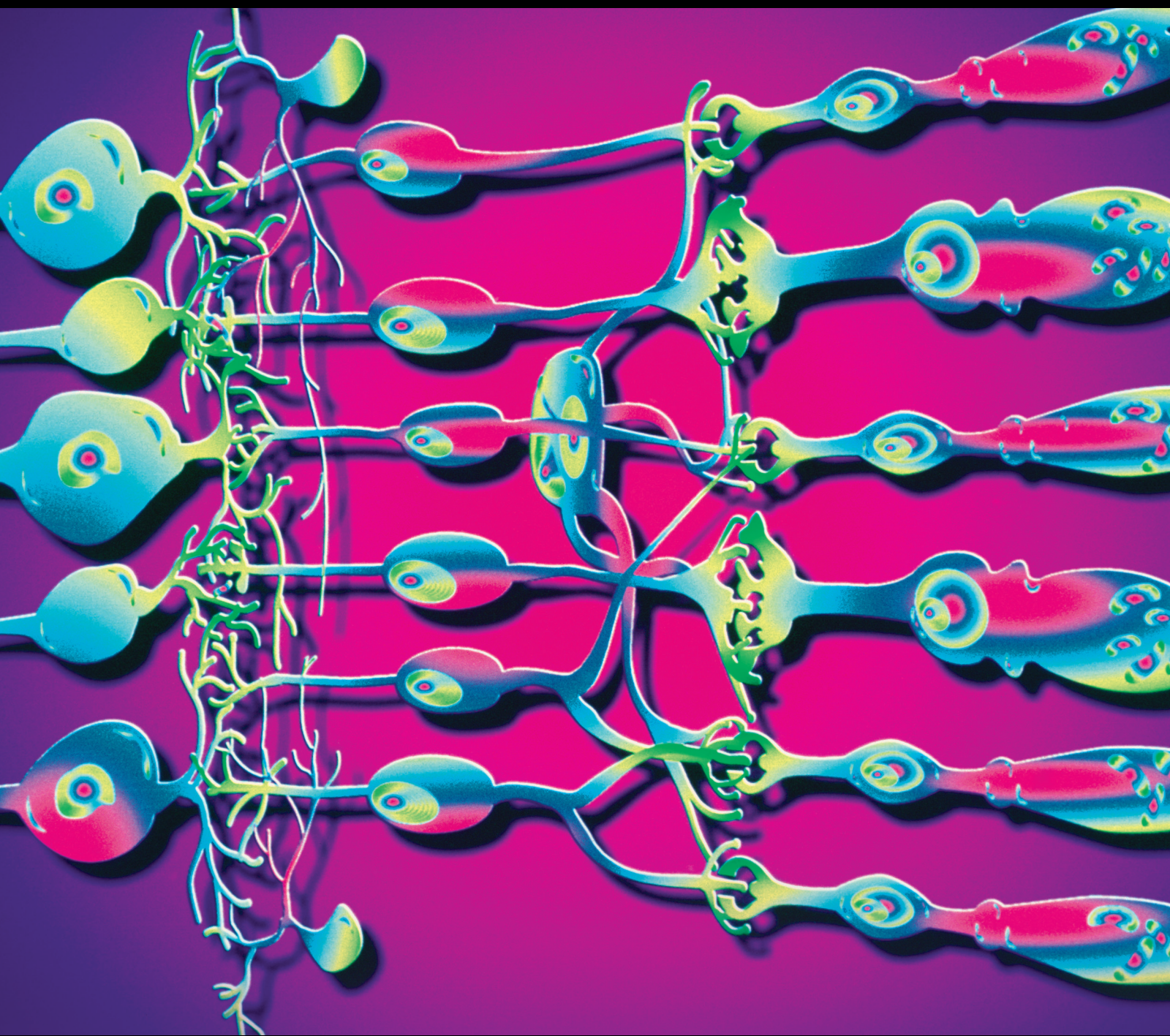
Journal of Ophthalmology

Lacrimal Gland, Ocular Surface, and Dry Eye

Guest Editors: Chuanqing Ding, Edit Tóth-Molnár, Ningli Wang, and Lei Zhou



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Editorial Board

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Editorial

Lacrimal Gland, Ocular Surface, and Dry Eye

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We hereby present to our readers what we have done in the past year, a special issue with 18 original research and review articles on lacrimal gland, ocular surface, and dry eye that covers wide topics in these areas.

As we have discussed in the call for papers, the main lacrimal gland, along with the ocular surface, which typically includes cornea, conjunctiva, meibomian gland, and other accessory glands, plays critical roles in the Ocular Surface System/Lacrimal Functional Unit. The interdependence and crosstalk among them are essential in maintaining the normal physiology and function of the ocular surface, providing essential nutrients, lubrication, and protection to the eyes to maintain their normal functions. Deficiencies in any of these tissues may lead to ocular surface diseases and vision impairment in its severe form.

There have seen enormous progress in our understanding of the components of the Ocular Surface System/Lacrimal Functional Unit since the new millennium. A quick PubMed search, using "Lacrimal Gland and Dry Eye" as key words, resulted in 1,459 publications tracing back to as early as 1946, with 919 of them published since 2000 (63% of the total). By using "Conjunctiva and Dry Eye" as key words, we found a total of 1,083 publications, with 740 of them published since 2000 (68% of the total; the earliest publication was in 1952). The main lacrimal gland is the major source of tear fluid, whereas in humans the conjunctiva occupies about 17 times more the surface area than the cornea. Increasing evidence suggests that both of them play essential roles in the etiology, progression, management, and prognosis of ocular surface diseases. The interactions among components of the Ocular Surface System/Lacrimal Functional Unit are vital to maintain the homeostasis of the ocular surface. Therefore it is critical to look at it as one unit in order to understand the pathogenesis of ocular surface diseases such as dry eye.

Dry eye is the most common reason for patient visits to the eye-care professionals, with epidemiology studies suggesting their prevalence ranging from 1-2% of the general population and can be as high as 30% in some groups, that is, seniors and women. Unfortunately, little is known about the etiology and pathogenesis of dry eye, and hence very limited management options are available at present. This further translates into enormous societal burden and economical losses, which has been estimated to be ~$55 billion/year in the United States alone, from this debilitating disease.

In this special issue, our authors have presented their findings and/or insights that span a wide range of topics, including the following: (i) novel polymer that may serve as potential eye drop excipient for treating dry eye; (ii) evaluation of the diagnostic value of McMonnies Questionnaire for dry eye screening in a multicenter study; (iii) exogenous hyaluronate playing a role in enhancing corneal epithelial cell wound healing; (iv) usefulness of measuring tear meniscus height and noninvasive keratograph tear breakup time as a simple and noninvasive screening test for dry eye patients; (v) high levels of 17β-estradiol being associated with increased

matrix metalloproteinase-2 and metalloproteinase-9 activity in the tears from postmenopausal women with dry eye; (vi) meibomian gland dysfunction playing a critical role in dry eye in myopic teenagers; (vii) short-term effect of air pollution on occurrence of nonspecific conjunctivitis; (viii) a useful rabbit dry eye model for assessing conjunctival functionality; (ix) diabetes mellitus affecting the quality and stability of tear film in many different ways; (x) a study demonstrating that hemodialysis is able to effectively reduce tear osmolarity to normal values; (xi) safety and effectiveness of the intense pulsed light for treating meibomian gland dysfunction; (xii) a study on the influence of septal deviation on the prognosis of transcanalicular diode laser-assisted dacryocystorhinostomy; (xiii) role of silicon tube intubation in transcanalicular multidiode laser dacryocystorhinostomy; (xiv) topical diquafosol versus cyclosporine in dry eye patients following cataract surgery.

While these studies may only represent a small percentage of publications in these topics, they surely provide some exciting progress made in the past year. We hope these novel findings and insights presented by our authors will be of help to other investigators in their research areas and instill new ideas for our eventual goal of finding the causes of dry eye and effective therapies for this debilitating disease that inflicts millions of people worldwide.

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Research Article

The Blockade of IL6 Counterparts the Osmolar Stress-Induced Apoptosis in Human Conjunctival Epithelial Cells

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To determine the effect of hyperosmolarity on cell survival/apoptosis of conjunctival epithelial cells and evaluate the possible role of IL6, Wong-Kilbourne derivative of Chang conjunctival cell line (WKD) was used in this study. Confluent cells were incubated under different osmolarity (290 mOsm and 500 mOsm) with or without neutralizing IL6 antibody (50 ng/mL). The expression of IL6 level was measured in the supernatant of each conditioned medium. Cell viability/apoptosis assay was performed using Annexin V/Propidium Iodide (PI) and Cell Counting Kit-8 (CCK-8). Western blot was conducted to measure the abundance of apoptotic markers and IL6 related downstream signaling pathway. The concentration of IL6 showed time-dependent increase in cells treated with 500 mOsm. Although apoptosis of WKD cell is increased in treated 500 mOsm for 24 h, apoptosis reduced in WKD cell treated 500 mOsm with anti-IL6 for 24 h. Anti-IL6 inhibited the activation of JAK-STAT signaling pathway, which was induced by hyperosmolarity. Hyperosmolar condition induced apoptosis in conjunctival epithelial cells, along with increase of IL6 production. IL6 neutralizing antibody inhibited apoptosis and JAK-STAT signaling in hyperosmolar condition. These findings suggested that IL6 may be involved in apoptotic change and in hyperosmolarity.

1. Introduction

Dry eye syndrome is a multifactorial disease, which is caused by a vicious cycle: abnormalities of tear film and lacrimal hyposecretion induce the break-up of tear film, and the following inflammation of ocular surface deteriorates the secretion and the composition of tears [1]. Hyperosmolarity is induced by lacrimal hyposecretion or the increase of evaporation evokes desquamation, decreased intercellular connections, blunting and loss of microplicae, cell membrane disruption, and cellular swelling with decreased cytoplasmic density in the corneal epithelium [2]. Moreover, hyperosmolarity provokes squamous metaplasia, loss of goblet cells, and inflammation in the conjunctival epithelium [3–5]. These phenomena decrease the production of mucin for lubricating corneal epithelium, and the reduction of mucin aggravates dry eye [6]. Histologic findings of dry eye in patients with Sjögren syndrome and immunosuppressant patients, such as postbone marrow transplantation state, were reported as a reduction of goblet cells, increment of inflammatory cells in cornea and conjunctiva, and inflammation with fibrosis of the lacrimal gland [7–10]. Dry eye also induces the secretion of cytokines such as IL6, IL-1β, TNF-α, and IFN-γ [11]. Cyclosporin, one of the drugs suppressing those cytokines, reduces the infiltration of conjunctival cells and IL6, regulates the necrosis of conjunctival epithelial cells, and elevates the number of goblet cells by preventing the loss of the cells [12].

IL6 has been known as a representative cytokine with increased expression in tears and the conjunctival epithelium of eyes with dry eye syndrome. It has also been reported to have pro- and anti-inflammatory effects. As evidence of the proinflammatory effects, a report revealed that IL6 treatment reduced the survival of liver cancer cells [13] and tocilizumab, an IL6 blocker, has been used as a therapeutic drug for autoimmune diseases in rheumatology. Anti-inflammatory effects of IL6 were revealed by the following evidence: IL6

treatment on cells increased migration [14], IL6 induced cell migration and wound healing of mouse biliary epithelial cells [15], blocking of IL6 reduced inflammatory-related molecules in mouse alkali burn model [16], and IL6-deficient mice showed delayed wound healing after skin resection [17].

Although IL6 has been well known as an important cytokine on disease progression and severity associated with immune mechanisms, its role in dry eye was still vague except for increased expression on the ocular surface. Therefore, this research investigated the role of IL6 on apoptosis of conjunctival epithelial cells after hyperosmolarity.

2. Material and Methods

2.1. In Vitro Osmolar Stress Experiment. The Wong-Kilbourne derivative of Chang conjunctival cells (WKD, ATCC CCL-20, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's Medium F12 (1 : 3) culture medium (Invitrogen, Waltham, MA, USA), supplemented with 1% penicillin and streptomycin (WELGENE, Daegu, Seoul) and 5% heat-inactivated fetal bovine serum (WISENT, Quebec, Canada). When cells were approximately 80–90% confluence, culture medium was replaced with fresh medium with added 1 M NaCl to increase the osmolarity (corresponding to 290, 500 mOsm) for 24 h. Cells were incubated for 24 h before protein extraction and conditioned medium collection.

The blockade of IL6 was done 24 h before incubation using the neutralizing Anti-Human IL6 antibody (anti-IL6, Clone 1936, R&D systems, Minneapolis, MN, USA) for neutralizing the IL6. To determine whether IL6 cytokine has protected effect in the NaCl exposure or not, conjunctiva epithelial cells were incubated with IL6 for 24 h and then exposed to 500 mOsm NaCl for 24 h.

2.2. Measurement of Cytokine Production. Cell supernatant was collected 290 mOsm, 500 mOsm NaCl incubation after 24 h, and then centrifuged 12,000 rpm for 3 min at 4°C. Cytokine levels in supernatant were determined using the Human IL-1β, IL6, TNF-α, and IFN-γ Quantikine Enzyme-Linked Immunosorbent Assay Kit (R&D systems, Minneapolis, MN, USA) and we followed the manufacturer's protocol. Briefly, for IL6 kit 100 μL of standard and samples were incubated in antibody-coated plate at 2 hrs. After being washed four times, add 200 μL conjugate solution being incubated at 2 hrs. After being washed four times, add 200 μL substrate solution being incubated at 20 min. Add 50 μL stop solution and read at 450 nm with reader within 30 min.

2.3. Cell Viability Assay. Cell viability was determined using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Cells (1×10^5 cells/mL, 100 μL) were seeded in 96-well plate. When cells were confluent 80%–90%, cells were treated with anti-IL6 for 24 h. After treatment for 24 h, add 10 μL of CCK-8 solution and incubate the plate for 1 h and cover the plate to protect from light, and then measure the absorbance using a microplate reader at 450 nm. The experiment was performed three times.

2.4. Flow Cytometry. Cells were seeded at 1.5×10^6 cells in a well of 60 mm plates and cultured until confluent 80–90%. Cells were exposed to 290 mOsm, 500 mOsm NaCl with/without anti-IL6 50 ng for 24 h. After 24 h, cells were collected and resuspended. For labeling the Annexin V/Propidium Iodide (PI), we were using the Annexin V/PI kit (Invitrogen, Eugene, Oregon, USA). The suspension cells were labeled with Annexin V/PI and analyzed by flow cytometry (BD, Franklin, New Jersey).

2.5. Western Blot Assay. Cells were harvested by scraping with RIPA buffer. Extracts were incubated for 2 h at 4°C and obtained by centrifugation (13,000 rpm for 20 min at 4°C). Protein concentrations were determined using the BCA assay Kit (Thermostat, Hercules, CA, USA), and whole-cell extracts were adjusted to same amount of total protein (20 μg). Samples were electrophoresed in 10% SDS-PAGE. Proteins were then transferred onto a PVDF membrane (Millipore Corporation, Billerica, MA, USA) at 300 mA for 90 min at 4°C, and the membranes were incubated with 3% BSA (Sigma-Aldrich, St. Louis, MO, USA) in TBST to block nonspecific binding. Primary antibodies were incubated over night at 4°C. And we washed five times with TBST (0.5% tween 20 in 1x TBS) the secondary antibodies conjugated horseradish peroxidase (HRP) (Santa Cruz, Dallas, Texas, USA) that was applied and incubated for 1 h at RT. After five times of washing with TBST (0.5% tween 20 in 1x TBS), the membrane followed by chemiluminescent detection using Immobilon Western Substrate (Millipore Corporation, Billerica, MA, USA) with the ChemiDoc MP Imaging system (Bio-Rad Laboratories Inc., Hercules, California, USA). The antibodies diluents were shown at Table 1.

2.6. Statistical Analysis. All results were indicated as means ± SEM. The results were analyzed by Kruskal-Wallis analysis, followed by a Mann-Whitney analysis. A p value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Hyperosmolarity Induces IL6 Levels in the WKD Cells. WKD cells were cultured under 290 mOsm and 500 mOsm for 24 h and the cell viability was analyzed. There were no definite apoptotic cell deaths in 290 mOsm, while cell viability was significantly reduced after 12 h in 500 mOsm (0.95 ± 0.04 at 12 h, 0.69 ± 0.012 at 24 h, and 0.47 ± 0.12 at 48 h, resp., $p = 0.05$ at 12 h, 24 h) (Figure 1(a)). We assessed the levels of IL-1β, TNF-α, IFN-γ, and IL6 from medium of cells cultured under 290 mOsm and 500 mOsm to identify the inflammatory cytokines which are well known to increase in epithelial cells by hyperosmolarity stress. Quantitative analysis showed that, whereas IL-1β (0.07 ± 0.002 in 290 mOsm, 0.06 ± 0.002 in 500 mOsm), TNF-α (0.08 ± 0.004 in 290 mOsm, 0.08 ± 0.0004 in 500 mOsm), and IFN-γ (0.08 ± 0.009 in 290 mOsm, 0.08 ± 0.003 in 500 mOsm) showed no significant change, IL6 (1.47 ± 0.44 in 290 mOsm, 3.23 ± 0.12 in 500 mOsm) were increased ($p = 0.05$, Figure 1(b)). When measuring the time-dependent expression of IL6 in 290 mOsm and 500 mOsm, the expression of IL6 in 500 mOsm increased

Table 1: List of antibodies, sources, and dilutions.

	Primary antibody	Host	Dilution	Manufacturer
JAK-STAT signaling markers	p-STAT3	Mouse	1 : 1000	Santa Cruz
	STAT3	Rabbit	1 : 1000	Santa Cruz
	p-ERK1/2	Rabbit	1 : 1000	Santa Cruz
	ERK1/2	Mouse	1 : 1000	Santa Cruz
	p-mTOR	Rabbit	1 : 1000	Santa Cruz
	mTOR	Goat	1 : 1000	Santa Cruz
Apoptosis markers	Bax	Rabbit	1 : 500	Santa Cruz
	Bcl2	Mouse	1 : 500	Santa Cruz
	Cleaved caspase-3	Rabbit	1 : 500	Abcam
	Caspase-3	Mouse	1 : 1000	Santa Cruz
Housekeeping gene marker	Beta-actin	Rabbit	1 : 4000	Abcam

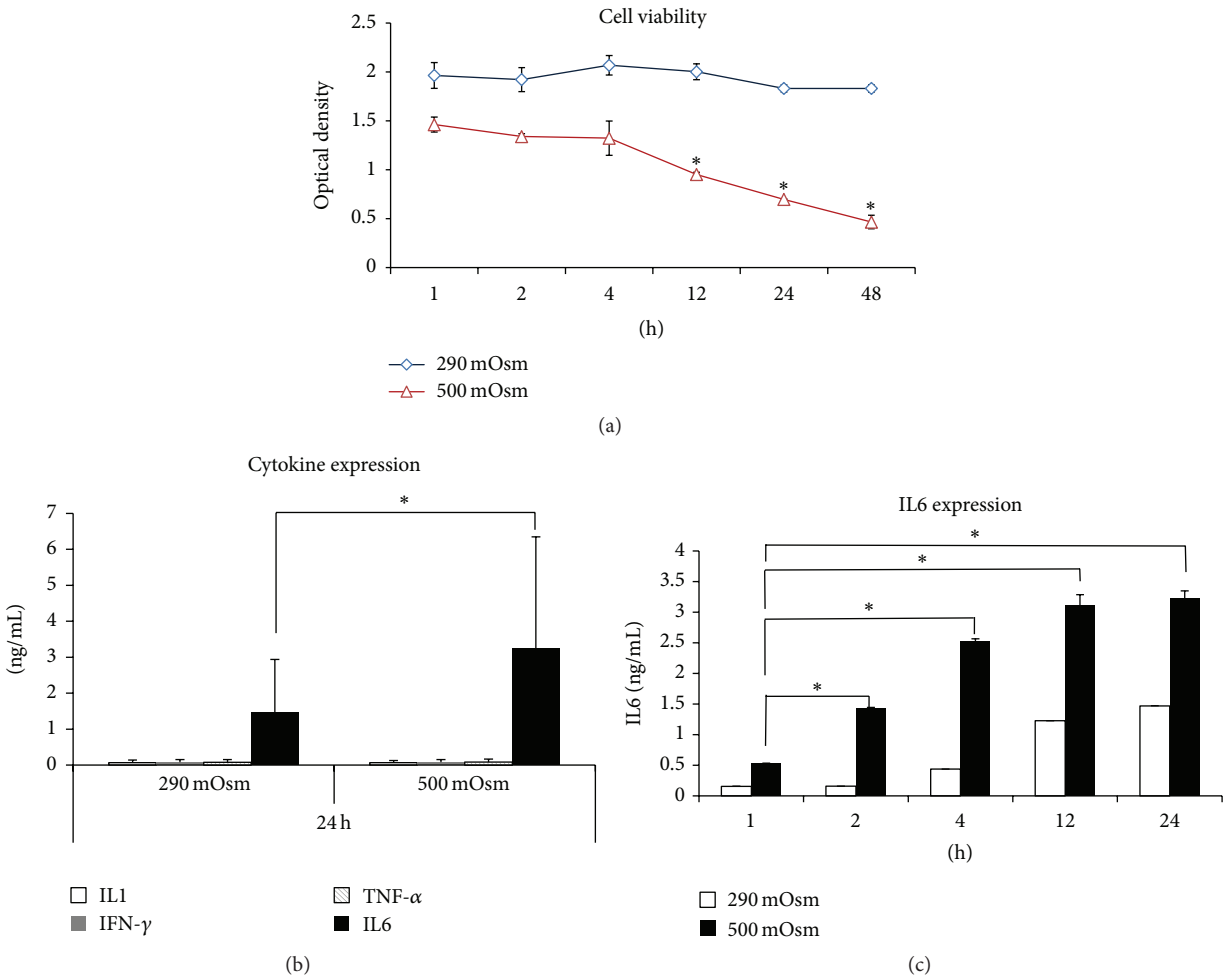

Figure 1: Change of conjunctival epithelial cells under 290 mOsm and 500 mOsm conditions. (a) Cell viability was significantly decreased in 500 mOsm at 12, 24, 48 h compared to 290 mOsm. (b) Cytokine ELISA assay showed 500 mOsm was significantly increased compared to 290 mOsm in cell supernatant. (c) IL6 increased depending on the time course, and the expression was higher in 500 mOsm than that in 290 mOsm. $^{*}p = 0.05$, $n = 3$.

from 0.012 pg/mL at 1 h to 2.1 pg/mL at 24 h, which was higher than that in 290 mOsm ($p = 0.05$, Figure 1(c)).

3.2. IL6 Mediates Hyperosmolarity Induced Apoptotic Cell Death. To evaluate whether IL6 was secreted to protect cells or induce cell death in apoptosis by hyperosmolarity, 50 ng of anti-IL6 was used for blocking IL6. When investigating change of morphology and cell distributions in 290 mOsm, 500 mOsm, and 500 mOsm with anti-IL6, the cells in 500 mOsm showed shrinkage and more apoptosis

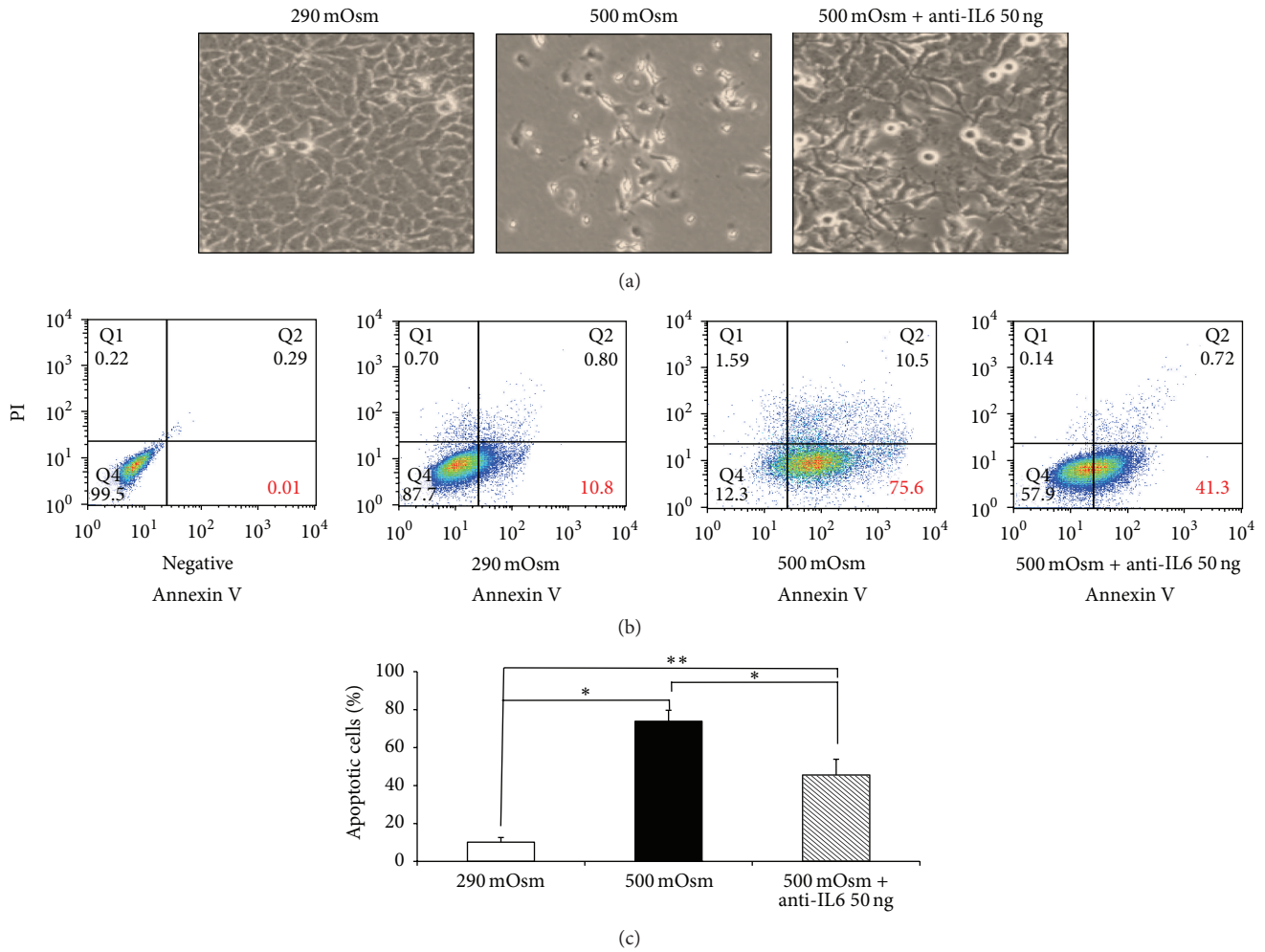

Figure 2: Effect of neutralizing IL6 antibody on cell apoptosis. (a) When evaluating the cell morphology, cell death decreased in 500 mOsm with anti-IL6 treatment. (b) Flow cytometry with Annexin V/PI showed reduced apoptosis under treatment of anti-IL6 and (c) the proportion of apoptotic cells significantly reduced by treatment of anti-IL6. $^{*}p = 0.022$, $^{**}p = 0.023$, $n = 3$.

than in 290 mOsm. Nevertheless, cells in 500 mOsm with anti-IL6 showed less apoptosis and shrinkage than those in 500 mOsm without anti-IL6 (Figure 2(a)).

We have given a 500 mOsm on the conjunctival epithelial cells and the effect of IL6 was examined using Annexin V/PI. The amount of the cells was significantly increased in the cell pretreated with the anti-IL6. The apoptosis rates are increased from 10.2 ± 2.44 in 290 mOsm to 73.88 ± 5.84 in 500 mOsm and 45.58 ± 2.89 in 500 mOsm NaCl with anti-IL6. Treated anti-IL6 was very effectively decreased for the apoptosis rates in 500 mOsm NaCl treated (Figure 2(b)).

There was significant difference in the proportions of apoptotic cells among 290 mOsm, 500 mOsm, and 500 mOsm with anti-IL6 (Figure 2(c)), which implies that IL6 could promote apoptosis by hyperosmolarity.

3.3. Anti-IL6 Inhibits JAK-STAT Pathway Activation in Hyperosmolarity Induced Cell Apoptosis. Evaluating JAK-STAT signaling and apoptosis markers by Western blot, the expressions of p-STAT3, p-ERK1/2, and p-mTOR were higher in 500 mOsm (resp., 1.01 ± 1.16, 1.19 ± 0.22, and 1.15 ± 0.08) than 290 mOsm (resp., 0.43±0.15, 0.44±0.08, and 0.48±0.03); however, those were lower in 500 mOsm with anti-IL6 (resp., 0.6 ± 0.17, 0.72 ± 0.17, and 0.9 ± 0.12) than in 500 mOsm.

Bax and cleaved-caspase-3 showed higher expression in 500 mOsm (resp., 1.39 ± 0.37, 1.08 ± 0.02) than in 290 mOsm (resp., 0.37 ± 0.17, 0.49 ± 0.14), and those were also lower in 500 mOsm with anti-IL6 (resp., 0.7 ± 0.2, 0.68 ± 0.18) than in 500 mOsm. Bcl2 showed lowest expression in 500 mOsm (0.3 ± 0.14), followed by that in 500 mOsm with anti-IL6 (0.66 ± 0.08) and 290 mOsm (1.24 ± 0.3) (Figure 3(a)). The significant difference of expressions of p-STAT3/total STAT3, p-ERK/total ERK, and p-mTOR/total mTOR was revealed between those in 500 mOsm and 500 mOsm with anti-IL6 ($p = 0.05$) Apoptosis markers such as Bax, Bcl2, and cleaved caspase-3/caspase-3 showed significant discrepancy between those in 500 mOsm and 500 mOsm with anti-IL6 ($p = 0.05$, Figure 3(b)).

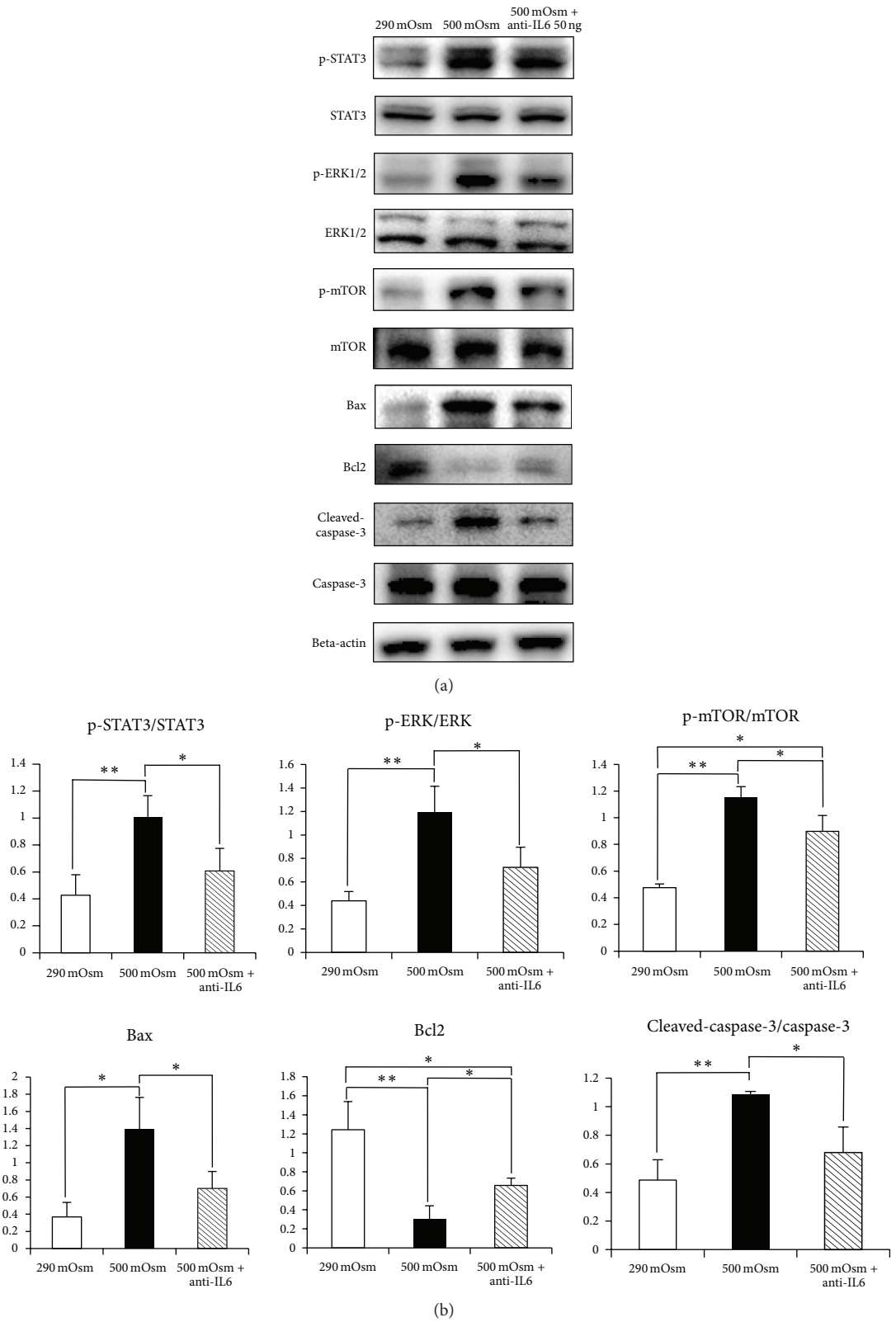

Figure 3: Effect of IL6 on JAK-STAT signaling and apoptosis markers. (a) The factors of the JAK-STAT signaling pathway and apoptosis markers in 500 mOsm and 500 mOsm with anti-IL6 were investigated by Western blot. JAK-STAT signaling was augmented in 500 mOsm and reduced in 500 mOsm with anti-IL6, and apoptosis markers also changed in similar pattern. (b) Those differences between 500 mOsm and 500 mOsm with anti-IL6 were statistically significant in the graph. $^{*}p = 0.05$, $^{**}p = 0.05$, $n = 3$.

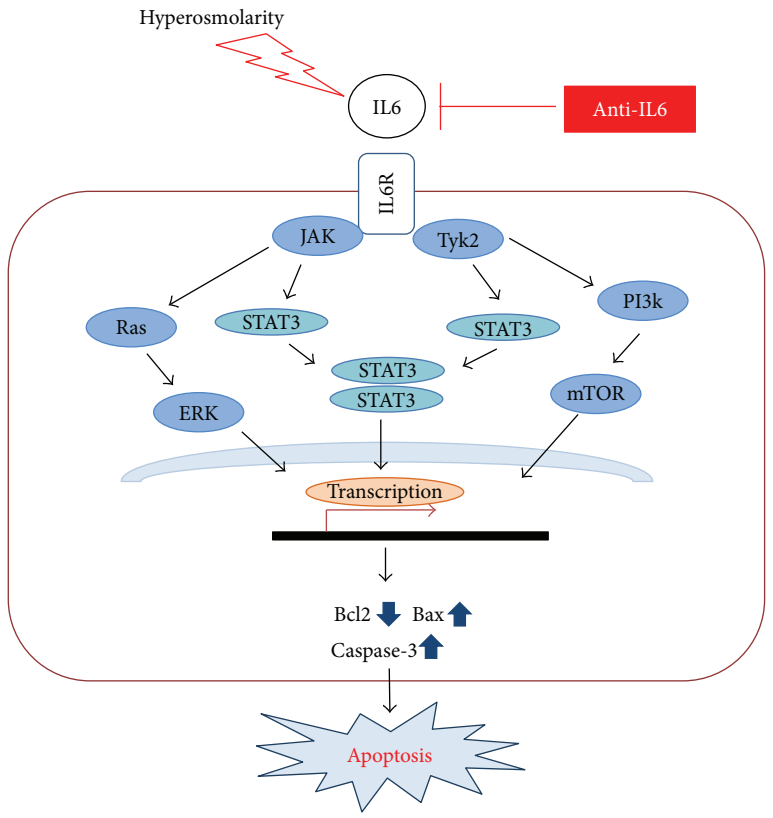

Figure 4: The role of IL6 induced by hyperosmolarity. Hyperosmolarity induced the expression of IL6 followed by apoptosis, whereas blocking IL6 suppressed JAK-STAT signaling and apoptosis.

4. Discussion

It has been reported that hyperosmolarity stress could induce apoptosis and promote the secretion of diverse proinflammatory cytokines [18]. The association between conjunctival epithelial cells and IL6 has been described in many studies: increased expression of IL-1β, IL8, IL6, and TNF-α in conjunctival epithelial cells and tear fluid in the patients with dry eye syndrome [19, 20] and increase of IL-1β, IL8, and, especially, IL6 in impression cytology [21, 22]. Those studies suggested conjunctiva epithelial cells could aggravate dry eye and IL6 had an important role in pathomechanism of dry eye. A previous study showed that blocking IL6 could increase goblet cells in conjunctival epithelium and reduce inflammatory cells, which could soothe the symptoms and prevent the chronic progression of dry eye [12]. Flow cytometry analysis showed that the blocking of IL6 significantly suppressed apoptosis observed at 500 mOsm, which suggests blocking IL6 could modify apoptosis following hyperosmolarity.

Cytokines such as IL6 can activate the JAK-STAT signaling pathway [23]. IL6 can be activated though the membrane-bound IL6 receptor (classical pathway) or the soluble type of IL6 receptor (trans-signaling). The IL6 attached with receptor could initiate cascade via JAK activation, and activated JAK kinase phosphorylate induces forming of dimer by phosphorylation of STAT3 [24, 25]. This signaling pathway promotes proliferation, differentiation, migration, and apoptosis of cells [26]. Although the activation of the JAK-STAT signal by IL6 was observed in cancer [27], there have been few studies for the association between conjunctival epithelial cells and IL6, especially about the role of increased IL6 in a hyperosmolar state of conjunctival epithelial cells. This research revealed that the IL6/JAK/STAT signaling pathway was associated with apoptosis induced by hyperosmolarity. Western blot showed that 500 mOsm promoted the expression of STAT3 (direct signal), ERK, and mTOR (indirect signal), and IL6 reduced the increased expressions. Bax and caspase-3 as apoptosis markers showed higher expression in hyperosmolar state (500 mOsm), which was suppressed by blocking IL6. The inverse pattern of Bcl-2 expression related to IL6 and hyperosmolarity was also revealed. These suggested that JAK-STAT signaling pathway of apoptosis was associated with IL6.

In this study, blocking IL6 inhibited the apoptosis of conjunctival epithelial cells under hyperosmolar condition, and the process was associated with the JAK-STAT signal pathway (Figure 4). This indicated that IL6 could be one of the important cytokines affecting the pathogenesis of dry eye. Therefore, in dry eye patients, IL6 could be a biologic marker, and regulating IL6 though the IL6/JAK/STAT3 signaling pathway could be an effective therapeutic target.

5. Conclusion

Hyperosmolarity induced apoptosis in conjunctival epithelial cells was suppressed by blocking IL6, which suggests that IL6

may play an important role in the pathogenesis of dry eye disease.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] C. Baudouin, P. Aragona, E. M. Messmer et al., "Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the OCEAN group meeting," *The Ocular Surface*, vol. 11, no. 4, pp. 246–258, 2013.

[2] J. P. Gilbard, J. B. Carter, D. N. Sang, M. F. Refojo, L. A. Hanninen, and K. R. Kenyon, "Morphologic effect of hyperosmolarity on rabbit corneal epithelium," *Ophthalmology*, vol. 91, no. 10, pp. 1205–1212, 1984.

[3] J. P. Gilbard, S. R. Rossi, K. L. Gray, L. A. Hanninen, and K. R. Kenyon, "Tear film osmolarity and ocular surface disease in two rabbit models for keratoconjunctivitis sicca," *Investigative Ophthalmology & Visual Science*, vol. 29, no. 3, pp. 374–378, 1988.

[4] J. P. Gilbard, S. R. Rossi, K. L. Gray, and L. A. Hanninen, "Natural history of disease in a rabbit model for keratoconjunctivitis sicca," *Acta Ophthalmologica*, vol. 67, supplement 192, pp. 95–101, 1989.

[5] S. C. Pflugfelder, S. C. G. Tseng, K. Yoshino, D. Monroy, C. Felix, and B. L. Reis, "Correlation of goblet cell density and mucosal epithelial membrane mucin expression with rose bengal staining in patients with ocular irritation," *Ophthalmology*, vol. 104, no. 2, pp. 223–235, 1997.

[6] I. K. Gipson and T. Inatomi, "Cellular origin of mucins of the ocular surface tear film," *Advances in Experimental Medicine and Biology*, vol. 438, pp. 221–227, 1998.

[7] W.-L. Fei, J.-Q. Chen, X. Du et al., "Dry eye in graft-versus-host disease," *Chinese Journal of Ophthalmology*, vol. 39, no. 11, pp. 686–690, 2003.

[8] Y. Ogawa, S. Okamoto, M. Wakui et al., "Dry eye after haematopoietic stem cell transplantation," *British Journal of Ophthalmology*, vol. 83, no. 10, pp. 1125–1130, 1999.

[9] T. Igarashi, C. Fujimoto, H. Suzuki et al., "Short-time exposure of hyperosmolarity triggers interleukin-6 expression in corneal epithelial cells," *Cornea*, vol. 33, no. 12, pp. 1342–1347, 2014.

[10] M. A. Lemp, C. Baudouin, J. Baum et al., "The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[11] K.-C. Yoon, I.-Y. Jeong, Y.-G. Park, and S.-Y. Yang, "Interleukin-6 and tumor necrosis factor-α levels in tears of patients with dry eye syndrome," *Cornea*, vol. 26, no. 4, pp. 431–437, 2007.

[12] K. Turner, S. C. Pflugfelder, Z. Ji, W. J. Feuer, M. Stern, and B. L. Reis, "Interleukin-6 levels in the conjunctival epithelium of patients with dry eye disease treated with cyclosporine ophthalmic emulsion," *Cornea*, vol. 19, no. 4, pp. 492–496, 2000.

[13] Y. Liu, P.-K. Li, C. Li, and J. Lin, "Inhibition of STAT3 signaling blocks the anti-apoptotic activity of IL-6 in human liver cancer cells," *The Journal of Biological Chemistry*, vol. 285, no. 35, pp. 27429–27439, 2010.

[14] I. A. Valsero, L. S. Romaní, L. G. Posadas, A. L. García, and Y. Diebold, "IL-6 as a corneal wound healing mediator in an invitro scratch assay," *Experimental Eye Research*, vol. 125, pp. 183–192, 2014.

[15] G.-X. Jiang, X.-Y. Zhong, Y.-F. Cui et al., "IL-6/STAT3/TFF3 signaling regulates human biliary epithelial cell migration and wound healing in vitro," *Molecular Biology Reports*, vol. 37, no. 8, pp. 3813–3818, 2010.

[16] T. Sakimoto, S. Sugaya, A. Ishimori, and M. Sawa, "Anti-inflammatory effect of IL-6 receptor blockade in corneal alkali burn," *Experimental Eye Research*, vol. 97, no. 1, pp. 98–104, 2012.

[17] Z.-Q. Lin, T. Kondo, Y. Ishida, T. Takayasu, and N. Mukaida, "Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice," *Journal of Leukocyte Biology*, vol. 73, no. 6, pp. 713–721, 2003.

[18] L. Luo, D.-Q. Li, and S. C. Pflugfelder, "Hyperosmolarity-induced apoptosis in human corneal epithelial cells is mediated by cytochrome c and MAPK pathways," *Cornea*, vol. 26, no. 4, pp. 452–460, 2007.

[19] S. C. Pflugfelder, D. Jones, Z. Ji, A. Afonso, and D. Monroy, "Altered cytokine balance in the tear fluid and conjunctiva of patients with Sjogren's syndrome keratoconjunctivitis sicca," *Current Eye Research*, vol. 19, no. 3, pp. 201–211, 1999.

[20] A. Solomon, D. Dursun, Z. Liu, Y. Xie, A. Macri, and S. C. Pflugfelder, "Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease," *Investigative Ophthalmology and Visual Science*, vol. 42, no. 10, pp. 2283–2292, 2001.

[21] D. T. Jones, D. Monroy, Z. Ji, S. S. Atherton, and S. C. Pflugfelder, "Sjogren's syndrome: cytokine and Epstein-Barr viral gene expression within the conjunctival epithelium," *Investigative Ophthalmology & Visual Science*, vol. 35, no. 9, pp. 3493–3504, 1994.

[22] J. Zhang, X. Yan, and H. Li, "Analysis of the correlations of mucins, inflammatory markers, and clinical tests in dry eye," *Cornea*, vol. 32, no. 7, pp. 928–932, 2013.

[23] D. Hebenstreit, J. Horejs-Hoeck, and A. Duschl, "JAK/STAT-dependent gene regulation by cytokines," *Drug News and Perspectives*, vol. 18, no. 4, pp. 243–249, 2005.

[24] R. J. Stefan, "Il-6 trans-signaling via the soluble IL-6 receptor: importance for the proinflammatory activities of IL-6," *International Journal of Biological Sciences*, vol. 8, no. 9, pp. 1237–1247, 2012.

[25] A. J. Brooks, W. Dai, M. L. O'Mara et al., "Mechanism of activation of protein kinase JAK2 by the growth hormone receptor," *Science*, vol. 344, no. 6185, Article ID 1249783, 2014.

[26] J. S. Rawlings, K. M. Rosler, and D. A. Harrison, "The JAK/STAT signaling pathway," *Cell Science*, vol. 117, pp. 1280–1283, 2004.

[27] S. C. Afford, J. Pongracz, R. A. Stockley, J. Crocker, and D. Burnett, "The induction by human interleukin-6 of apoptosis in the promonocytic cell line U937 and human neutrophils," *The Journal of Biological Chemistry*, vol. 267, no. 30, pp. 21612–21616, 1992.

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Research Article

Evaluation of the Safety and Effectiveness of Intense Pulsed Light in the Treatment of Meibomian Gland Dysfunction

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Purpose. This study aims to explore the safety and efficacy of a novel treatment-intense pulsed light (IPL) in MGD eyes. *Methods.* This study is a prospective and open label study. Forty eyes of 40 MGD patients were recruited in the study and received 4 consecutive IPL treatments on day 1, day 15, day 45, and day 75. Ten ocular surface symptoms were evaluated with a subjective face score at every visit. Best spectacle corrected visual acuity, intraocular pressure (IOP), conjunctival injection, upper and lower tear meniscus height (TMH), tear break-up time (TBUT), corneal staining, lid margin and meibomian gland assessments, and meibography were also recorded at every visit, as well as the adverse effects on the eye and ocular surface. *Results.* Significant improvements were observed in single and total ocular surface symptom scores, TBUT, and conjunctival injection at all the visits after the initial IPL treatment ($P < 0.05$). Compared to baseline, the signs of eyelid margin, meibomian gland secretion quality, and expressibility were significantly improved at every visit after treatments. There was no regional and systemic threat observed in any patient. *Conclusion.* Intense pulsed light (IPL) therapy is a safe and efficient treatment in relieving symptoms and signs of MGD eyes.

1. Introduction

Meibomian Gland Dysfunction (MGD) is one of the most common causes of dry eye [1]. It is a diffuse deformity of the meibomian glands, whose terminal duct is fully or partly obstructed. The glandular secretion is changed in quality or/and quantity [1], which results in an unstable tear film. Its main symptoms range from dryness, eye irritation, foreign body sensation, burning, and watering to fatigue [2]. The prevalence of MGD varies broadly worldwide, from 3.5% to nearly 70% [3], which is of concern to clinical doctors and scientists.

The pathogenesis of MGD begins with ductal epithelium hyperkeratinization and increased meibum viscosity. The obstruction subsequently happens when the terminal duct is filled with thickened opaque meibum which contains keratinized cell material, resulting in intraglandular cystic dilation, gland dropout, and low secretion [4, 5]. Reduced meibum outflow will boost the proliferation of commensal bacteria [6, 7], releasing fatty acids and mono- and diglycerides into the tear film causing a sense of irritation [8, 9].

The treatments of MGD vary from artificial tears, warm compression [10–13], meibomian gland expression [14–16], and omega-3 supplementation [17] to cyclosporine [18], corticosteroids, and oral antibiotics [19], all of which have been shown to provide only short-term symptom relief [2, 20]. This suggests that we need more treatment options, one of which is intense pulsed light therapy. Intense pulsed light (IPL) therapy is generally used in the cosmetic industry [21] for disease like benign cavernous hemangiomas, telangiectasia, port-wine stains, and so forth [21–25]. IPL treatment applies Xenon flash lamp to emitting wavelengths of light ranging from 400 to 1200 nm, and various chromophores (hemoglobin, melanin, and water) will be targeted concurrently [26].

The initial application of intense pulsed light for dry eye patients began in 2002 by Dr. Rolando Toyos when a patient with rosacea indicated improvement of dry-eye symptoms after receiving IPL treatment [27–30]. Since then, studies about the treatment of dry eye syndrome caused by MGD by IPL have gradually shown its benefits [31, 32]. The prevalence of MGD in Asian populations (>60%) is much higher than

that of Caucasians (3.5%–19.9%) [4]. Due to the differences of the pigmentation and skin type between Chinese and Caucasian, the efficacy and safety of the IPL wavelengths might be diverse. While similar studies in Chinese patients in this field are rather few, the efficacy, safety, and mechanism of this new therapy in Chinese patients needs further evaluation.

Our study aimed to collect the data of IPL therapy in Chinese MGD patients so that we can clarify the effectiveness and safety of IPL therapy in Chinese MGD patients. The IPL device we used is currently the only certified IPL device (E-Eye; E-SWIN, Paris, France) for treating MGD [31]. The wavelength of the device ranges from the lower visible spectrum (580 nm) to near infrared (1200 nm).

2. Materials and Methods

2.1. Patients. The present study was conducted according to the principles of the Declaration of Helsinki and was approved by the Human Research and Ethics Committee of Peking University Third Hospital. Written informed consent form was obtained from each participant before enrolment. This study is a prospective and open label study.

Subjects were recruited from the outpatient department of the Department of Ophthalmology of Peking University Third Hospital between April 2014 and January 2015. The inclusion criteria for this study were (1) adult patients; (2) chief complaint of one of the following symptoms: dryness, foreign body sensation, burning, and tearing for more than 3 months; (3) diagnosis of MGD with two or more of the following signs in both eyes: redness or thickening of the lid margin, telangiectasia, reduced or no secretions, poor quality secretions, and gland capping [33]; (4) willingness to cooperate with the doctors in the follow-up visits. Exclusion criteria included patients with (1) severe ocular surface abnormalities; (2) history of ocular trauma or surgery; (3) punctal occlusion; (4) use of any eye drops other than artificial tears within the past 1 month; (5) active allergy or infection or inflammatory disease at the ocular surface unrelated to dry eye or MGD; (6) current use of treatments for MGD; (7) alterations of the lacrimal drainage system, (8) use of systemic medications altering the tear film; (9) contact lens wear; (10) systemic diseases affecting the ocular surface; (11) uncontrolled systemic disease; (12) pigmented lesions in the treatment area; (13) skin treatments within 2 months; (14) pregnancy/nursing mothers.

Forty eyes (left eyes were selected at random) of 40 MGD patients (18 males and 22 females) were enrolled into this prospective study, with a mean age of 51.3 ± 20.1 years (ranged 21–78 years).

2.2. Treatment Procedure. With an E-Eye machine provided by E-SWIN company, France (https://www.e-swin.com/), IPL treatment was administered to the skin area below the lower eyelid [31]. Before treatment, the eyes were protected with opaque goggles and ultrasound gel was applied on the patient's face from tragus to tragus including the nose to conduct the light, help to spread the energy evenly, and provide a degree of protection [32]. The intensity of the IPL treatment ranges from 9.8 J/cm^2 to 13 J/cm^2 in accordance with the Fitzpatrick Skin Type Grading [31]. In each IPL treatment, 4 overlapping flashes were applied to the skin area below the lower eyelid for every eye with no pressure. All the treatment areas were identical within different subjects. The subjects received four separate treatment sessions on day (D) 1, D15, D45, and D75. All the treatments were performed by the same doctor (JXD).

2.3. Clinical Evaluation. Subjects were evaluated at four visits: 3 days before the first IPL treatment and 15 days, 45 days, 75 days after the first treatment. The clinical assessments of the subject and both eyes were carried out in the following order: symptoms evaluation, best spectacle corrected visual acuity, intraocular pressure (IOP), conjunctival injection, upper and lower tear meniscus height (TMH), tear break-up time (TBUT), corneal staining, lid margin and meibomian gland assessments, and meibography. An interval of 5 minutes was required between different tests. All the test data were collected by two doctors (LHB and ZMZ); the average would be defined as the final results. All the evaluations and assessments were carried out before the IPL treatment at each visit.

2.4. Symptom Evaluation. The severity of the following 10 ocular symptoms for every subject was assessed by the ophthalmologist at clinic visits on baseline, day 15, day 45, and day 75: dryness, foreign body sensation, watering, itchiness, visual fatigue, blurred vision, burning, sensitivity to light, secretion disturbance, and pain. For each symptom assessment, subjective face scores were applied [20]. 11 faces were shown to the subjects, with the saddest face (score 10) representing the most severe discomfort and the happiest face (score 0) describing no discomfort. A total subjective symptom score was defined as the summation of these scores; thus minimum of score was 0, and maximum was 100.

2.5. Intraocular Pressure (IOP). IOP of every eye was measured by noncontact tonometer (Canon TX-20, Japan) with no topical anaesthetic used, which is not invasive. The procedure was evaluated for three times, and the average value was defined as the final score.

2.6. Conjunctival Injection and TMH. Conjunctival injection degree was evaluated under slit lamp microscope. Institute for Eye Research (IER) Grading Scales [34] were used to assess bulbar redness with score 0 representing grade 1 and score 3 meaning grade 4, which describe severe redness of the bulbar conjunctiva. The central upper and lower TMH were measured by a slit lamp microscope (with a graticule in 0.05 mm units) [35]. Three consecutive readings were evaluated and the median was defined as the final results.

2.7. TBUT and Corneal Staining. A total of 5 μL of 2% sodium fluorescein was instilled onto the bulbar conjunctiva without inducing reflex tearing, by using a micropipette [36]. The patient was asked to blink naturally without squeezing for three to five times, and then the patient was asked to stare straight ahead without blinking, until told otherwise, under

the cobalt blue light [36]. A stopwatch was used to record the time between the last complete blink and the first appearance of a dry spot or disruption in the tear film [36]. The procedure was evaluated for three times, and the average value was defined as the final score. For corneal staining evaluation, the cornea was divided into five sectors [37]; each sector was graded from 0 to 3 using the following criteria: 0 no staining; 1 punctate/stipple staining; 2 ball and linear staining; and 3 coalesced staining [38].

2.8. Eyelid Margin and Meibomian Gland Assessments. According to the International Workshop on Meibomian Gland Dysfunction, five signs of eyelid margin were assessed in our study: rounding of posterior margin, irregularity/notching of margin, telangiectasia/vascularity of lid margin, trichiasis, and anterior blepharitis. Each sign scored 0 or 1. Score 0 equals no/normal; score 1 equals yes/abnormal. Meibomian gland assessments included (1) the average of the number of the upper and lower present lid orifices; (2) expressed secretion quality; (3) the expressibility of the meibomian gland. The levels of the quality were divided into 4 degrees: 0 = clear; 1 = cloudy; 2 = granular; 3 = toothpaste, as well as the expressibility: 1 = light; 2 = moderate; 3 = heavy pressure [39].

2.9. Safety Evaluation. At every visit, best spectacle corrected visual acuity, intraocular pressure (IOP), and corneal and conjunctival examinations by slit lamp microscope were performed. Eyelash abnormities such as eyelash loss and trichiasis were evaluated by slit lamp microscope. The assessments of the skin area around the eye were also carried out for the examination of depigmentation, blistering, swelling, redness, and hair loss at brow and forehead.

2.10. Statistical Analysis. Statistical analysis was performed by using R software (Version 2.14.2). Comparison between data points was performed with paired t-test with the Bonferroni correction, which compared the single and total symptoms, conjunctival injection, TMH, TBUT, corneal staining, and the number of meibomian gland orifices at the three different follow-up times to those of the last visits. Chi square test was used to compare the eyelid margin signs, meibomian gland secretions, and expressibility at three different follow-up times to those of last visits.

3. Results

The full cohort of 40 enrolled participants completed measurements across all four appointments and were included in the analysis.

3.1. Clinical Symptoms. Ten ocular surface symptoms were evaluated at every visit and the results were listed in Table 1. The symptom scores were collected before the treatment with IPL at every visit. Compared to baseline, all symptoms were significantly relieved at the time of D15, D45, and D75 ($P < 0.05$) except blurred vision. Between the visits of D15 and D45, significant relief was continuously observed in dryness ($P < 0.01$) and pain ($P = 0.03$). However, between the visits of D45 and D75, there was no significant difference among all symptoms. The total score of the 10 single symptoms was defined as the total symptom scores (Figure 1). Compared to baseline, the total symptom score significantly decreased at the time of D15, D45, and D75 ($P < 0.01$). Between the visits of D15 and D45, the total score continuously decreased ($P = 0.04$), while between the visits of D45 and D75, no significant difference was observed ($P = 1$).

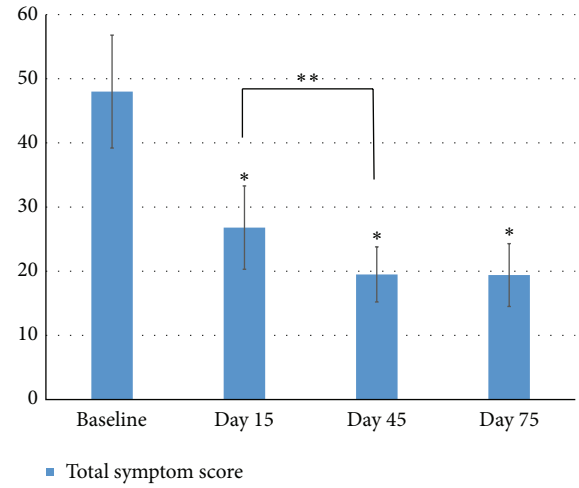

Figure 1: Total symptom scores. Notes: the total scores of the 10 single symptoms were defined as the total symptom scores. *Compared to the baseline, the total symptom score significantly decreased at the time of D15, D45, and D75 ($P < 0.01$). **Between the visits of D15 and D45, the total score continuously decreased ($P = 0.04$), while between the visits of D45 and D75, no significant difference was observed ($P = 1$). Statistical analysis was performed with paired t-test with the Bonferroni correction.

3.2. Eyelid Margin and Meibomian Gland Assessments. Five signs of eyelid margin, rounding of posterior margin, irregularity/notching of margin, telangiectasia/vascularity of lid margin, trichiasis, and anterior blepharitis, were evaluated at every visit and recorded in Table 2. Compared to baseline, all the signs except trichiasis were significantly improved after the treatments ($P < 0.05$). The number of the meibomian gland orifices within the central 1 cm was significantly increased at D15 (4.3 ± 3.1), D45 (5.3 ± 3.5), and D75 (4.9 ± 3.3), compared to those from baseline ($P < 0.01$). Between the visits of D15 and D45, the number continuously increased ($P = 0.02$), while between the visits of D45 and D75, no significant difference was observed ($P = 0.96$). Compared to the baseline, the meibomian gland secretion quality and expressibility significantly improved at the visit of D15 ($P < 0.05$) and continuously improved at the visit of D45, which was compared to those at D15 ($P < 0.05$) (Figure 2). Between the visits of D45 and D75, no significant difference was observed for the secretion quality ($P = 0.68$) and expressibility ($P = 0.29$) (Figure 2).

3.3. TBUT and Corneal Staining. TBUT at D15 (4.2 ± 1.8), D45 (5.0 ± 1.9), and D75 (4.5 ± 2.5) were significantly

Table 1: 10 ocular surface symptoms' evaluation during IPL treatments.

Symptoms	Mean ± SD						
	Baseline	D15	P^{**}	D45	P^{***}	D75	P^{****}
Dryness	9.5 ± 2.2	5.9 ± 3.5	<0.01*	4.1 ± 3.1	<0.01*	4.2 ± 3.0	1
Foreign body sensation	8.0 ± 4.1	4.8 ± 3.9	<0.01*	3.5 ± 3.4	0.129	3.7 ± 3.2	1
Itching	3.8 ± 4.9	1.8 ± 3.4	0.03	1.1 ± 2.6	0.54	1.0 ± 2.6	1
Burning	3.8 ± 4.9	1.1 ± 2.8	<0.01*	0.9 ± 2.5	1	1.3 ± 2.8	0.93
Visual fatigue	5.0 ± 5.1	3.5 ± 4.0	0.01*	2.8 ± 3.4	0.24	2.5 ± 3.4	1
Blurred vision	1.0 ± 3.1	0.7 ± 2.2	0.54	0.3 ± 1.5	1	0.2 ± 0.9	1
Sensitivity to light	4.3 ± 5.0	2.3 ± 3.4	<0.01*	1.8 ± 3.1	0.45	1.6 ± 2.8	1
Watering	2.5 ± 4.4	1.1 ± 2.8	0.02*	0.8 ± 2.4	1	0.7 ± 1.9	1
Secretion disturbance	5.8 ± 5.0	2.8 ± 3.3	<0.01*	2.7 ± 3.4	1	2.6 ± 3.8	1
Pain	4.3 ± 5.0	2.8 ± 3.9	0.04*	1.5 ± 2.7	0.03*	1.6 ± 3.1	1

Notes: * $P < 0.05$; ** compared to baseline; *** compared to D15; **** compared to D45. Statistical analysis was performed with paired t-test with the Bonferroni correction.
SD: standard deviation; D: day.

Table 2: Evaluation of eyelid margin signs during IPL treatments.

Eyelid margin	n (%)				P^{**}
	Baseline	Day 15	Day 45	Day 75	
Rounding of posterior margin	29 (72.5%)	22 (55.0%)	16 (40.0%)	14 (35.0%)	<0.01*
Irregularity	23 (57.5%)	12 (30.0%)	6 (15.0%)	6 (15.0%)	<0.01*
Telangiectasia	26 (65.0%)	20 (50.0%)	11 (27.5%)	6 (15.0%)	<0.01*
Trichiasis	1 (2.5%)	1 (2.5%)	0 (0%)	3 (7.5%)	0.27
Anterior blepharitis	38 (95.0%)	35 (87.5%)	30 (75.0%)	28 (70.0%)	0.01*

Notes: * $P < 0.05$; ** Chi square test.

increased compared to that at the baseline (2.2 ± 1.5) ($P < 0.01$) (Figure 3). Between the visits of D15 and D45, TBUT continuously increased while reaching no statistic difference ($P = 0.07$). Between the visits of D45 and D75, no significant difference was observed ($P = 0.51$). No significant difference was found in the assessment of corneal staining among all visits (Table 3).

3.4. Conjunctival Injection and TMH. Compared to baseline, conjunctival injection was significantly relieved at D15, D45, and D75 ($P = 0.01$). Among the visits of D15, D45, and D75, no significant difference was observed (Table 3). No significant difference was found in the assessment of upper and lower TMH among all visits (Figure 3).

3.5. Safety Data. Among all visits, best spectacle corrected visual acuity was not significantly changed; IOPs of all subjects were lower than 21 mmHg. There was no depigmentation, blistering, swelling, redness, and hair loss at the brown and ocular surface. There was no significant eyelash loss during the evaluation, either. No systemic adverse event was observed during the study.

4. Discussion

Meibomian Gland Dysfunction (MGD) is a high prevalent ocular surface disease. The efficacy of conventional treatment for MGD remains to be transient and unsatisfactory, suggesting the need for the exploration of new therapeutic approaches. Our study applied IPL treatment to the skin around the eyes in 40 Chinese MGD patients (40 eyes) and provided a strong evidence for the effectiveness and safety of IPL treatment in relieving ocular surface symptoms and signs.

In our research, a series of 10 comprehensive subjective self-reported symptoms associated with MGD were evaluated with a face scorecard. All symptoms except blurred vision significantly improved after the initial IPL treatment. Furthermore, two symptoms, dryness and pain, continuously relieved significantly after the second IPL treatment, as well as the total symptom scores. The improvement of the single and total symptom scores remains steady after the visits of D45 to D75, which implied that twice IPL treatments might meet the maximum therapeutic effects in relieving ocular surface symptoms. For a long time, MGD associated dry eye has been also considered as a chronic pain disease [38]. Much research indicates that chronic inflammatory status of MGD or dry eye is able to lower the pain threshold and increase neurogenic sensitivity through proinflammatory factors [40].

Table 3: Evaluation of number of orifices within central 1 cm, conjunctival injection, and corneal staining changes during IPL treatments.

	Mean ± SD			
	Baseline	Day 15	Day 45	Day 75
Number of orifices (central 1 cm)	2.8 ± 2.8	4.3 ± 3.1	5.3 ± 3.5	4.9 ± 3.3
P^{**}		<0.01*	0.02*	0.96
Conjunctival injection	0.95 ± 0.45	0.60 ± 0.55	0.58 ± 0.55	0.63 ± 0.49
P^{**}		0.01*	1	1
Corneal staining	0.15 ± 0.49	0.03 ± 0.16	0.03 ± 0.16	0.08 ± 0.35
P^{**}		0.39	1	0.96

Notes: *$P < 0.05$; **paired t-test compared to the previous visit with the Bonferroni correction.
SD: standard deviation.

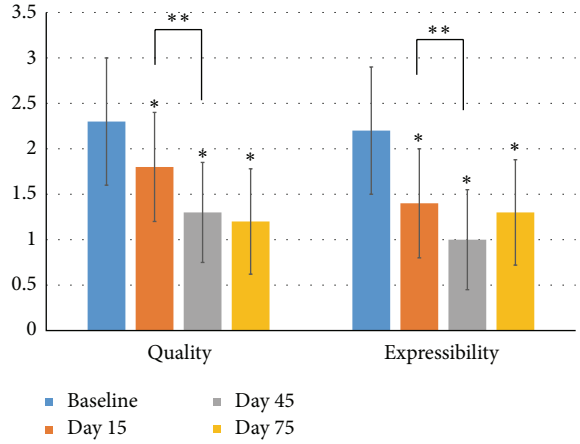

Figure 2: Meibomian gland secretion quality and expressibility. Notes: the y axis represents the level or grade of the meibomian gland secretion quality and expressibility, with higher grades meaning worse quality and expressibility. *Compared to the baseline, the meibomian gland secretion quality and expressibility significantly improved at the visits of D15, D45, and D75 ($P < 0.05$). **Between the visits of D15 and D45, the meibomian gland secretion quality and expressibility continuously improved ($P < 0.05$). Between the visits of D45 and D75, no significant difference was observed for the secretion quality ($P = 0.68$) and expressibility ($P = 0.29$). Statistical analysis was performed with paired t-test with the Bonferroni correction.

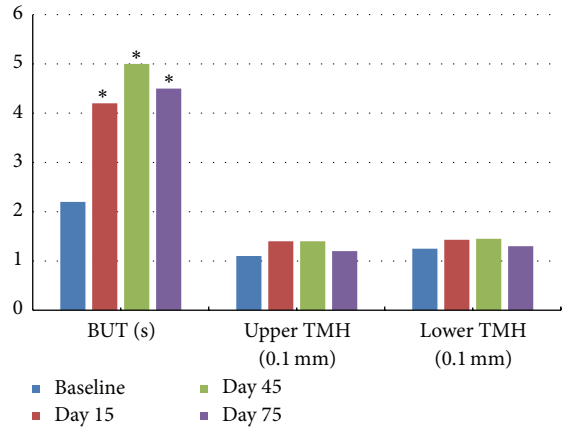

Figure 3: Tear break-up time (TBUT) and tear meniscus height (TMH). Notes: *TBUT at D15 (4.2 ± 1.8 s), D45 (5.0 ± 1.9 s), and D75 (4.5 ± 2.5 s) were significantly increased compared to that at the baseline (2.2 ± 1.5 s) ($P < 0.01$). Between the visits of D15 and D45, TBUT continuously increased while reaching no statistic difference ($P = 0.07$). Between the visits of D45 and D75, no significant difference was observed ($P = 0.51$). No significant difference was found in the assessment of upper and lower TMH in all visits. Statistical analysis was performed with paired t-test with the Bonferroni correction.

Wavelength of 600–950 nm which is included in our IPL treatment is proved to be effective in relief of inflammation pain and neurogenic sensitivity [41, 42]. Our study also showed that the ocular pain was relieved significantly after the IPL treatment. One of the mechanisms of symptoms relief effect may relate to the neurogenic sensitivity and pain adjustments of IPL therapy. Blurred vision in MGD and dry eye patients are mainly caused by shortened BUT, reaching no significant change in our study. Even though the BUT increased after IPL treatment, the BUT at every visit was still far away from normal (>10 s), which may resulted in the blurred vision symptom. On the other hand, the blurred vision symptom did not aggravate which implied the safety profile that the IPL treatment exerted no influence on the vision acuity.

Five signs of the eyelid margin: rounding of posterior margin, irregularity/notching of margin, telangiectasia/vascularity of lid margin, trichiasis, and anterior blepharitis were evaluated in our study. Rounding of posterior margin, irregularity, telangiectasia, and anterior blepharitis experienced great improvements. Numerous studies showed that hemoglobin primarily absorbs at a wavelength of 580 nm [43] and then causes the blood cells in the abnormal telangiectasias to absorb the light, to coagulate, and, finally, to close the blood vessels, thus improving vascularization [32]. Our study showed similar effects of IPL treatment: eyelid telangiectasia were significantly relieved, as well as the conjunctival injection. Such improvements in telangiectasia may prevent inflammatory mediator secretion and decrease bacterial overgrowth [32].

The alteration of meibomian gland secretion quality and expressibility is the key characteristics in MGD eyes. Our

research revealed significant improvements of meibomian gland secretion quality and expressibility after IPL treatments. Similar results were observed in Goto et al.'s research [20], who applied an infrared warm compression device to the meibomian gland. Studies [3] showed that melting point of meibomian gland secretions in subjects with MGD was 3°C higher than that in normal eyes and thermal therapies such as warm compression were able to melt the pathologically dysfunctional lipids and relieve the ocular surface symptoms associated with MGD. Theoretically, the light coming from IPL device is directly exposed to the skin and could result in a production of heat higher than body temperature [31], which is enough to melt the pathological secretion. During the IPL treatment, enough ultrasound gel should be used on the patient's face from tragus to tragus including the nose to conduct the light, help to spread the energy evenly, and provide a degree of protection.

Our study also found that TBUT was significantly lengthened after IPL treatment. Tear film is a highly organized structure on the ocular surface; its stability and function are highly relied on its biochemical composition [44]. The improvement of the meibomian gland secretion quality and expressibility by IPL treatment may have a direct effect on the stability of tear film. Craig and colleagues [31] found out that IPL therapy was able to improve the lipid layer grade in tear film. The presumed decrease of proinflammatory factors arising from the decreased the eyelid telangiectasia and conjunctival injection relief observed in our study may also play a role in the stability of tear film.

Overall, there are some possible mechanisms whereby IPL treatment could relieve ocular surface symptoms and signs of MGD eyes. First, IPL is able to produce a heat effect which melts the pathologically dysfunctional secretions. Second, the IPL device we applied emits energy in a band from a base of the visible spectrum (580 nm) to near infrared (1200 nm), which can be absorbed by hemoglobin, causing the thrombosis of the abnormal vascular in eyelid margin and related conjunctiva. Third, IPL treatment may exert an effect in relief of inflammation pain and neurogenic pain [41, 42], which is highly related to the improvement of clinical symptoms.

There are some limitations in our study including the following. (1) The first is lack of control group. The compressing effect of the goggles worn during the IPL may exert a role, which should be ruled out; lacking a nontreatment control group, the placebo effect and the risk of investigator bias could have influenced the results. Further studies should be carried out with placebo controls or positive controls to rule out the above influences. (2) The second is reporting subjective symptoms in an open label study. Reporting subjective symptoms which would be considered a low level of scientific evidence was applied in our study. (3) The short time of observation was limited to 75 days; the final treatment was performed at the very day as the final evaluation which suggested that the full effect of the final treatment might not have been realised. A follow-up is also needed after treatment termination to assess the long-term effectiveness and safety of such treatment. (4) TMH measurement technique is too insensitive (with a graticule in 0.05 mm units) to reveal differences. A better technique should be applied for better sensitivity. (5) Mechanisms of IPL treatment in MGD eyes were not proven in our study. Further research should be carried out to explore the exact mechanisms or molecular changes during IPL therapy in MGD eyes.

5. Conclusion

Meibomian Gland Dysfunction (MGD) is one of the most common causes of dry eye, resulting in a range of symptoms including dryness, burning, foreign body sensation, and blurred vision. The treatments of MGD, which are numerous, remain to be inefficient and not comprehensive. IPL treatment is a newly advanced choice for MGD patients. Our study applied a consecutive IPL treatment to Chinese MGD patients and demonstrated that it was able to relieve the symptoms and signs of MGD in Chinese patients safely and effectively, which may open up a potential new treatment for MGD.

Disclosure

Xiaodan Jiang and Huibin Lv both contributed as first authors.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

References

[1] K. K. Nichols, "The international workshop on meibomian gland dysfunction: introduction," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 4, pp. 1917–1921, 2011.

[2] J. L. Gayton, "Etiology, prevalence, and treatment of dry eye disease," *Clinical Ophthalmology*, vol. 3, no. 1, pp. 405–412, 2009.

[3] D. A. Schaumberg, J. J. Nichols, E. B. Papas, L. Tong, M. Uchino, and K. K. Nichols, "The international workshop on meibomian gland dysfunction: report of the subcommittee on the epidemiology of, and associated risk factors for, MGD," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 4, pp. 1994–2005, 2011.

[4] K. K. Nichols, G. N. Foulks, A. J. Bron et al., "The international workshop on meibomian gland dysfunction: executive summary," *Investigative Ophthalmology and Visual Science*, vol. 52, no. 4, pp. 1922–1929, 2011.

[5] G. N. Foulks and A. J. Bron, "Meibomian gland dysfunction: a clinical scheme for description, diagnosis, classification, and grading," *Ocular Surface*, vol. 1, no. 3, pp. 107–126, 2003.

[6] D. Borchman, G. N. Foulks, M. C. Yappert, and S. E. Milliner, "Differences in human meibum lipid composition with meibomian gland dysfunction using NMR and principal component analysis," *Investigative Ophthalmology & Visual Science*, vol. 53, no. 1, pp. 337–347, 2012.

[7] J. M. Dougherty and J. P. McCulley, "Bacterial lipases and chronic blepharitis," *Investigative Ophthalmology & Visual Science*, vol. 27, no. 4, pp. 486–491, 1986.

[8] J. E. Graham, J. E. Moore, X. Jiru et al., "Ocular pathogen or commensal: a PCR-based study of surface bacterial flora in

normal and dry eyes," *Investigative Ophthalmology and Visual Science*, vol. 48, no. 12, pp. 5616–5623, 2007.

[9] M. Berry, A. Harris, R. Lumb, and K. Powell, "Commensal ocular bacteria degrade mucins," *The British Journal of Ophthalmology*, vol. 86, no. 12, pp. 1412–1416, 2002.

[10] M. C. Olson, D. R. Korb, and J. V. Greiner, "Increase in tear film lipid layer thickness following treatment with warm compresses in patients with meibomian gland dysfunction," *Eye & Contact Lens*, vol. 29, no. 2, pp. 96–99, 2003.

[11] P. S. Bilkhu, S. A. Naroo, and J. S. Wolffsohn, "Effect of a commercially available warm compress on eyelid temperature and tear film in healthy eyes," *Optometry and Vision Science*, vol. 91, no. 2, pp. 163–170, 2014.

[12] A. Mori, J. Shimazaki, S. Shimmura, H. Fujishima, Y. Oguchi, and K. Tsubota, "Disposable eyelid-warming device for the treatment of meibomian gland dysfunction," *Japanese Journal of Ophthalmology*, vol. 47, no. 6, pp. 578–586, 2003.

[13] D. R. Korb and C. A. Blackie, "Restoration of meibomian gland functionality with novel thermodynamic treatment device—a case report," *Cornea*, vol. 29, no. 8, pp. 930–933, 2010.

[14] J. P. Craig, K. Blades, and S. Patel, "Tear lipid layer structure and stability following expression of the meibomian glands," *Ophthalmic and Physiological Optics*, vol. 15, no. 6, pp. 569–574, 1995.

[15] D. R. Korb and C. A. Blackie, "Meibomian gland therapeutic expression: quantifying the applied pressure and the limitation of resulting pain," *Eye and Contact Lens*, vol. 37, no. 5, pp. 298–301, 2011.

[16] D. R. Korb and C. A. Blackie, "Meibomian gland diagnostic expressibility: correlation with dry eye symptoms and gland location," *Cornea*, vol. 27, no. 10, pp. 1142–1147, 2008.

[17] A. Oleñik, I. Mahillo-Fernández, N. Alejandre-Alba et al., "Benefits of omega-3 fatty acid dietary supplementation on health-related quality of life in patients with meibomian gland dysfunction," *Clinical Ophthalmology*, vol. 8, pp. 831–836, 2014.

[18] L. Yang, Z. Yang, H. Yu, and H. Song, "Acupuncture therapy is more effective than artificial tears for dry eye syndrome: evidence based on a meta-analysis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 143858, 11 pages, 2015.

[19] B. Sobolewska, D. Doycheva, C. Deuter, I. Pfeffer, M. Schaller, and M. Zierhut, "Treatment of ocular rosacea with once-daily low-dose doxycycline," *Cornea*, vol. 33, no. 3, pp. 257–260, 2014.

[20] E. Goto, Y. Monden, Y. Takano et al., "Treatment of non-inflamed obstructive meibomian gland dysfunction by an infrared warm compression device," *British Journal of Ophthalmology*, vol. 86, no. 12, pp. 1403–1407, 2002.

[21] M. Haedersdal, F. Beerwerth, and J. F. Nash, "Laser and intense pulsed light hair removal technologies: from professional to home use," *British Journal of Dermatology*, vol. 165, no. 3, pp. 31–36, 2011.

[22] M. P. Goldman, R. A. Weiss, and M. A. Weiss, "Intense pulsed light as a nonablative approach to photoaging," *Dermatologic Surgery*, vol. 31, no. 9, pp. 1179–1187, 2005.

[23] R. C. R. Patriota, C. J. Rodrigues, and L. C. Cucé, "Intense pulsed light in photoaging: a clinical, histopathological and immunohistochemical evaluation," *Anais Brasileiros de Dermatologia*, vol. 86, no. 6, pp. 1129–1133, 2011.

[24] Y.-H. Li, Y. Wu, J. Z. Chen et al., "Application of a new intense pulsed light device in the treatment of photoaging skin in Asian patients," *Dermatologic Surgery*, vol. 34, no. 11, pp. 1459–1464, 2008.

[25] K. Negishi, Y. Tezuka, N. Kushikata, and S. Wakamatsu, "Photorejuvenation for Asian skin by intense pulsed light," *Dermatologic Surgery*, vol. 27, no. 7, pp. 627–632, 2001.

[26] D. J. Goldberg, "Current trends in intense pulsed light," *The Journal of Clinical and Aesthetic Dermatology*, vol. 5, no. 6, pp. 45–53, 2012.

[27] R. Toyos, *Intense Pulsed Light for Dry Eye Syndrome*, Cataract & Refractive Surgery Today, 2009.

[28] S. T. Tan, A. Bialostocki, and J. R. Armstrong, "Pulsed dye laser therapy for rosacea," *British Journal of Plastic Surgery*, vol. 57, no. 4, pp. 303–310, 2004.

[29] C. A. Schroeter, S. Haaf-Von Below, and H. A. M. Neumann, "Effective treatment of rosacea using intense pulsed light systems," *Dermatologic Surgery*, vol. 31, no. 10, pp. 1285–1289, 2005.

[30] P. Papageorgiou, W. Clayton, S. Norwood, S. Chopra, and M. Rustin, "Treatment of rosacea with intense pulsed light: significant improvement and long-lasting results," *British Journal of Dermatology*, vol. 159, no. 3, pp. 628–632, 2008.

[31] J. P. Craig, Y.-H. Chen, and P. R. K. Turnbull, "Prospective trial of intense pulsed light for the treatment of meibomian gland dysfunction," *Investigative Ophthalmology and Visual Science*, vol. 56, no. 3, pp. 1965–1970, 2015.

[32] R. Toyos, W. McGill, and D. Briscoe, "Intense pulsed light treatment for dry eye disease due to meibomian gland dysfunction; A 3-Year Retrospective Study," *Photomedicine and Laser Surgery*, vol. 33, no. 1, pp. 41–46, 2015.

[33] P. A. Asbell, F. J. Stapleton, K. Wickström et al., "The international workshop on meibomian gland dysfunction: report of the clinical trials subcommittee," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 4, pp. 2065–2085, 2011.

[34] B. Severinsky, D. Wajnsztajn, and J. Frucht-Pery, "Silicone hydrogel mini-scleral contact lenses in early stage after corneal collagen cross-linking for keratoconus: a retrospective case series," *Clinical and Experimental Optometry*, vol. 96, no. 6, pp. 542–546, 2013.

[35] H. Pult, C. Purslow, and P. J. Murphy, "The relationship between clinical signs and dry eye symptoms," *Eye*, vol. 25, no. 4, pp. 502–510, 2011.

[36] "Introduction to the Report of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 69–70, 2007.

[37] B. E. Caffery and J. E. Josephson, "Corneal staining after sequential instillations of fluorescein over 30 days," *Optometry and Vision Science*, vol. 68, no. 6, pp. 467–469, 1991.

[38] M. L. Thomas, V. R. Szeto, C. M. Gan, and K. A. Polse, "Sequential staining the effects of sodium fluorescein, osmolarity, and pH on human corneal epithelium," *Optometry and Vision Science*, vol. 74, no. 4, pp. 207–210, 1997.

[39] A. Tomlinson, A. J. Bron, D. R. Korb et al., "The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 4, pp. 2006–2049, 2011.

[40] X.-M. Wang, M. Hamza, T.-X. Wu, and R. A. Dionne, "Upregulation of IL-6, IL-8 and CCL2 gene expression after acute inflammation: correlation to clinical pain," *Pain*, vol. 142, no. 3, pp. 275–283, 2009.

[41] C. H. Leal de Godoy, P. F. da Costa Silva, D. S. de Araujo et al., "Evaluation of effect of low-level laser therapy on adolescents with temporomandibular disorder: study protocol for a randomized controlled trial," *Trials*, vol. 14, no. 1, article 229, 2013.

[42] J. Irvine, S. L. Chong, N. Amirjani, and K. M. Chan, "Double-blind randomized controlled trial of low-level laser therapy in carpal tunnel syndrome," *Muscle & Nerve*, vol. 30, no. 2, pp. 182–187, 2004.

[43] S. G. Fabi and M. P. Goldman, "The safety and efficacy of combining poly-L-lactic acid with intense pulsed light in facial rejuvenation: a retrospective study of 90 patients," *Dermatologic Surgery*, vol. 38, no. 7, part 2, pp. 1208–1216, 2012.

[44] A. Puinhas, P. Sampaio, E. M. S. Castanheira, M. E. C. D. Real Oliveira, and M. Lira, "Comparison of IgA, TNF-α and surface tension of the tear film in two different times of the day," *Contact Lens and Anterior Eye*, vol. 36, no. 3, pp. 140–145, 2013.

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Research Article

The Effect of Air Pollution on the Occurrence of Nonspecific Conjunctivitis

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Purpose. To investigate the short-term effect of air pollution on occurrence of nonspecific conjunctivitis. *Methods.* Data were collected from outpatient visits from cases with conjunctivitis over a period of one year. Regression analysis was performed to evaluate the relationship between the number of outpatient visits and the air quality and the lag effect of air quality on conjunctivitis occurrence. *Results.* The air quality index on the day of presentation ($P = 0.023$), one day before presentation ($P = 0.049$), and two days before presentation day ($P = 0.050$) had a positive relation with outpatient visits for conjunctivitis. The air quality index ($P = 0.001$) and outpatient visits number per day ($P = 0.013$) in autumn and winter (October to March) were significantly higher than those in spring (April) and summer (September). *Conclusions.* The air quality index within two days before presentation affected the probability of attending the outpatient clinic for nonspecific conjunctivitis. High number of cases can be expected in colder season.

1. Introduction

Air pollution is a risk factor for various diseases including eye irritation, respiratory infections, and heart disease [1–3]. Conjunctiva is sensitive to environmental particles considering the direct contact of conjunctiva with the outside environment [4]. Conjunctiva protects the ocular from outsides deleterious agents, helps lubricate the eye by producing mucus and tears, and contributes to the immune balance of ocular surface. The importance of conjunctiva and a high prevalence of conjunctivitis merit an investigation on the effect of air pollutant on conjunctivitis.

The environmental pollution, especially the air quality, has deteriorated in the past decades in China mainly due to the rapid industrialization in the country [5]. The maximal air quality index can reach above 500 in some parts of China. Overall, no more than 5 cities among the 500 largest cities of China meet the air quality guidelines recommended by the World Health Organization. Recently, seven cities in China were ranked among the 10 most polluted cities in the world [6]. The current study aims to evaluate the effect of air pollution on the occurrence of nonspecific conjunctivitis through analyzing the patients diagnosed as nonspecific conjunctivitis in Jinan city and the air pollution level of Jinan city.

2. Methods

Data was collected from two eye centers in Jinan city: central area and east area of Shandong Provincial Hospital, Shandong University. Patients presenting to the outpatients clinic between June 2014 and May 2015 with symptoms and signs of nonspecific conjunctivitis were included. Outpatient visits for nonspecific conjunctivitis were selected according to a previously published report [7] and the International Classification of Diseases (ICD-9) diagnostic codes. The following codes were included: 372.00, 372.01, 372.10, 372.11, 372.20, and 372.30 (for nonspecific acute conjunctivitis, serious conjunctivitis except viral infection, chronic conjunctivitis, simple chronic conjunctivitis, blepharoconjunctivitis, and other undefined conjunctivitis, resp.). The following cases

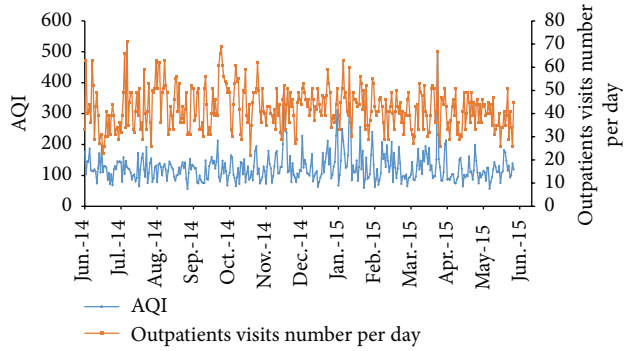

Figure 1: A total of 15373 patients were enrolled in this study from June 2014 to May 2015, and the AQI was recorded within same interval. The average patients number per day and AQI were 42 (22–71) and 125 (56–500), respectively.

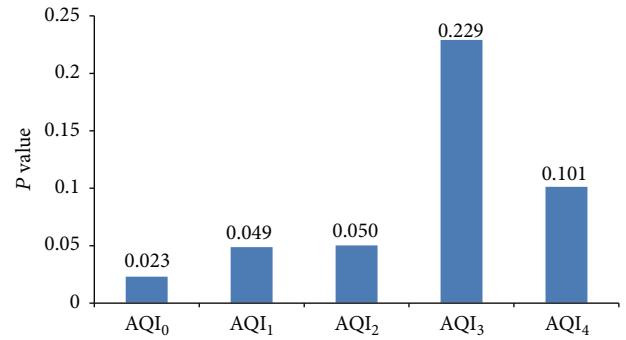

Figure 2: The patients number per day has positive relation with AQI_0 ($P = 0.023$), AQI_1 ($P = 0.049$), and AQI_2 ($P = 0.050$), but not AQI_3 ($P = 0.229$) and AQI_4 ($P = 0.101$).

were excluded: patients with other ocular diseases including corneal abnormalities, conjunctivitis before the initiation of the study, xerophthalmia, and systemic immune disease.

Air pollution data was harvested from the State Environmental Protection Administration of China and expressed as air quality index (AQI). The AQI was composed by the index of particulate matter (PM_{10} and $PM_{2.5}$), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone (O_3), and carbon monoxide (CO).

The linear regression analysis was used to evaluate the relationship between number of clinic visits per day and AQI from the same day up to 4 prior days. The AQI on presenting day was expressed as AQI_0. The AQI within 1 day, 2 days, 3 days, and 4 days were calculated as the mean of the AQI on presenting day and 1 day, 2 days, 3 days, and 4 days prior to presentation and were expressed as AQI_1, AQI_2, AQI_3, and AQI_4. Statistical analysis was performed with SPSS (version 16.0 for Windows). $P < 0.05$ was considered as statistically significant.

3. Results

A total of 15373 patients living in the air-quality-monitoring area of Jinan city were enrolled in this study. The average number of patients with nonspecific conjunctivitis per day was 42 (22–71), and the average AQI was 125 (56–500) (Figure 1).

The AQI_0 ($P = 0.023$), AQI_1 ($P = 0.049$), and AQI_2 ($P = 0.050$) had a positive relation with the number of patients per day (Figure 2). However, the AQI_3 ($P = 0.229$) and AQI_4 ($P = 0.101$) did not have a significant relation with patient numbers per day (Figure 2). The AQI ($P = 0.001$) as well as the number of patients per day ($P = 0.013$) in autumn and winter (October to March) was higher compared to that in spring and summer (April and September).

4. Discussion

In the present study, the AQI was harvested from 15 areas of Jinan district covering 3000 km^2 and 4 million people. Previous studies have demonstrated the effect of air pollution on respiratory disorders [8, 9]. A similar reaction to exogenous stimuli between conjunctival mucosa and respiratory mucosa has been proposed in the past [10, 11]. Chang et al. [7] reported a positive relation between air pollution and outpatient visits for nonspecific conjunctivitis in Taiwan area. The different components of air pollutants have different effects on the occurrence of conjunctivitis [7]. In present study, we reported that the occurrence of conjunctivitis has positive relation with the AQI on presenting day and the AQI within one day before the day of presentation. A limitation of our study is that we did not investigate the effect of different components of pollutants on causation of conjunctivitis. Present study observed a variety of conjunctivitis types within ICD-9 code but did not predefine various forms of infections and allergic or physiological changes in tear film disorders except with the ICD codes. More study should be done to elucidate the correlation between these various types of conjunctivitis and the various air quality measurements that were monitored.

Present study revealed that the AQI in autumn and winter is higher than that in spring and summer. The same trend was observed in the number of outpatient visits. The effect of temperature and humidity on conjunctivitis should also be considered besides AQI. A high AQI in autumn and winter in Jinan may be due to more coal consumption for heating, use of firecrackers consumption from spring festival to lantern festival, and a more difficult spread of pollutants due to low temperature.

This study was carried out in an area with heavy air pollution, in which a variety of health disorders are related to pollutants. Although present study has revealed a relation between air pollution and conjunctivitis, more detailed investigations should be carried out to elucidate the effect of age and sex on the ophthalmic response to pollutant and the clinical treatment. Furthermore, the relationship between conjunctivitis and dry eye [12, 13] merits the investigation of effect of air pollution on dry eye and other more severe ophthalmic disorders related to dry eyes dry eye, such as microbial keratitis [14] and the decline in quality of life [15].

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Zhiwei Li and Xiaoyan Bian are co-first authors who have equal contribution to present study.

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References

[1] S. E. Eftim, J. M. Samet, H. Janes, A. McDermott, and F. Dominici, "Fine particulate matter and mortality: a comparison of the six cities and American Cancer Society cohorts with a Medicare cohort," *Epidemiology*, vol. 19, no. 2, pp. 209–216, 2008.

[2] C. A. Pope III, "Air pollution and health-good news and bad," *New England Journal of Medicine*, vol. 351, no. 11, pp. 1132–1134, 2004.

[3] C. A. Pope III and D. W. Dockery, "Health effects of fine particulate air pollution: lines that connect," *Journal of the Air and Waste Management Association*, vol. 56, no. 6, pp. 709–742, 2006.

[4] I. S. J. Tuominen, Y. T. Konttinen, M. H. Vesaluoma, J. A. O. Moilanen, M. Helintö, and T. M. T. Tervo, "Corneal innervation and morphology in primary Sjögren's syndrome," *Investigative Ophthalmology & Visual Science*, vol. 44, no. 6, pp. 2545–2549, 2003.

[5] X. Y. Zhang, Y. Q. Wang, T. Niu et al., "Atmospheric aerosol compositions in China: spatial/temporal variability, chemical signature, regional haze distribution and comparisons with global aerosols," *Atmospheric Chemistry and Physics*, vol. 12, no. 2, pp. 779–799, 2012.

[6] Toward an Environmentally Sustainable Future: Country Environmental Analysis of the People's Republic of China. Asian Development Bank, 2013, http://www.adb.org/publications/toward-environmentally-sustainable-future-country-environmental-analysis-prc.

[7] C.-J. Chang, H.-H. Yang, C.-A. Chang, and H.-Y. Tsai, "Relationship between air pollution and outpatient visits for nonspecific conjunctivitis," *Investigative Ophthalmology and Visual Science*, vol. 53, no. 1, pp. 429–433, 2012.

[8] M. Brauer, G. Hoek, P. Van Vliet et al., "Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children," *American Journal of Respiratory and Critical Care Medicine*, vol. 166, no. 8, pp. 1092–1098, 2002.

[9] B. Oftedal, B. Brunekreef, W. Nystad, C. Madsen, S.-E. Walker, and P. Nafstad, "Residential outdoor air pollution and lung function in schoolchildren," *Epidemiology*, vol. 19, no. 1, pp. 129–137, 2008.

[10] L. Bielory and M. H. Friedlaender, "Allergic conjunctivitis," *Immunology and Allergy Clinics of North America*, vol. 28, no. 1, pp. 43–58, 2008.

[11] S. J. Ono and M. B. Abelson, "Allergic conjunctivitis: update on pathophysiology and prospects for future treatment," *Journal of Allergy and Clinical Immunology*, vol. 115, no. 1, pp. 118–122, 2005.

[12] M. M. Hom, A. L. Nguyen, and L. Bielory, "Allergic conjunctivitis and dry eye syndrome," *Annals of Allergy, Asthma and Immunology*, vol. 108, no. 3, pp. 163–166, 2012.

[13] H. Uchida and Y. Imanaga, "Effect of mild conjunctivitis complication on tear balance in dry eye," *Contact Lens and Anterior Eye*, vol. 35, no. 5, pp. 240–242, 2012.

[14] S. Narayanan, R. L. Redfern, W. L. Miller, K. K. Nichols, and A. M. Mcdermott, "Dry eye disease and microbial keratitis: is there a connection?" *Ocular Surface*, vol. 11, no. 2, pp. 75–92, 2013.

[15] M. Li, L. Gong, W. J. Chapin, and M. Zhu, "Assessment of vision-related quality of life in dry eye patients," *Investigative Ophthalmology and Visual Science*, vol. 53, no. 9, pp. 5722–5727, 2012.

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Research Article

Cationic Thiolated Poly(aspartamide) Polymer as a Potential Excipient for Artificial Tear Formulations

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Dry eye disease is a relatively common ocular problem, which causes eye discomfort and visual disorders leading to a decrease in the quality of life. The aim of this study was to find a possible excipient for eye drop formulations, which is able to stabilize the tear film. A cationic thiolated polyaspartamide polymer, poly[(*N*-mercaptoethylaspartamide)-co-(*N*-(*N*′,*N*′-dimethylaminoethyl)aspartamide)] (ThioPASP-DME), was used as a potential vehicle. Besides satisfying the basic requirements, the chemical structure of ThioPASP-DME is similar to those of ocular mucins as it is a protein-like polymer bearing a considerable number of thiol groups. The solution of the polymer is therefore able to mimic the physiological properties of the mucins and it can interact with the mucus layer via disulphide bond formation. The resultant mucoadhesion provides a prolonged residence time and ensures protective effect for the corneal/conjunctival epithelium. ThioPASP-DME also has an antioxidant effect due to the presence of the thiol groups. The applicability of ThioPASP-DME as a potential excipient in eye drops was determined by means of ocular compatibility tests and through examinations of the interactions with the mucosal surface. The results indicate that ThioPASP-DME can serve as a potential eye drop excipient for the therapy of dry eye disease.

1. Introduction

Dry eye disease (DED) has been reported to afflict 7–33% of the population, thereby reducing their quality of life. For normal vision, continuous moistening of the ocular surface is needed. Important roles are played in this by a sufficient quality of tears, maintenance of the normal composition of the tear film, normal lid closure, and regular blinking [1, 2]. If equilibrium is lost, the DED can occur, resulting in eye discomfort and visual disturbance [2, 3].

DED is accompanied by changes in mucin distribution and glycosylation, a dysfunction of MUC4 and MUC5AC and a high calcium level [4]. The mucins act as a lubricant during blinking, stabilize the preocular tear film to prevent desiccation of the epithelium, and form a barrier against pathogen penetrance [5]. Intracellular calcium is responsible for cationic shielding to keep negatively charged mucins condensed and packed within the granules of goblet cells. In the event of enhanced calcium release, the granules swell, become detached from the cell surface, form large aggregates, and diffuse onto the epithelial surface. This leads to a lower degree of hydration of the mucus coverage, which contains dry spots, resulting in decreased tear film stability [4].

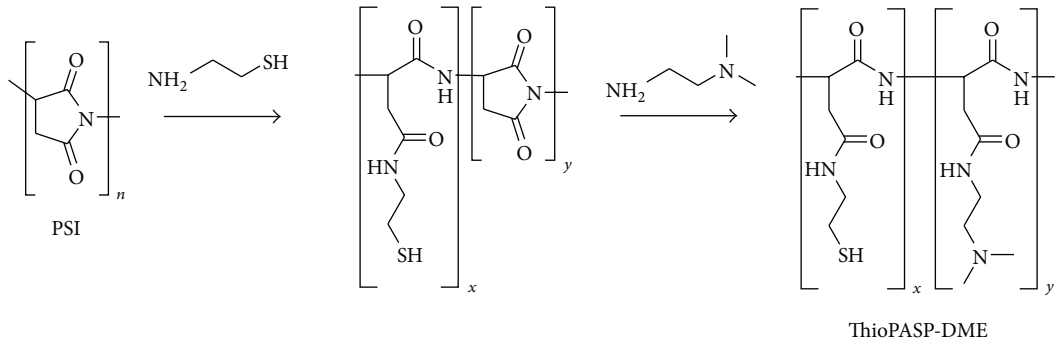

Figure 1: Synthesis of cationic ThioPASP-DME polymers.

One way to stabilize the tear film in cases of DED is to use liquid thiolated polymer formulations, whose structures are similar to those of ocular mucins, as they are protein-like polymers bearing a considerable number of thiol groups. The solutions of such polymers are therefore able to mimic the physiological properties of mucins, such as tear film stabilization. The formation of disulphide bonds with the mucus layer leads to strong mucoadhesion, which may be further strengthened by the formation of ionic bonds between the cationic groups of the excipient and the anionic groups of the mucins. The strong adhesion promotes a prolonged residence time and a protective effect for the corneal/conjunctival epithelium. Liquid formulations also serve as lubricants, prolonging the breakup time of the tear film. Moreover, thiolated polymers have antioxidant and radical scavenging properties and can therefore be useful excipients in artificial tear formulations for the therapy of DED [4, 6].

We earlier described thiolated poly(aspartic acid) (ThioPASP) polymers, which are biocompatible [7, 8], *in situ* gelling, and potential ophthalmic vehicles [9, 10]. The aims of the present study were to synthetize and characterize a cationic thiolated poly(aspartamide) bearing both cationic tertiary amine and redox-responsive thiol pendant groups as a potential mucoadhesive and tear film-stabilizing excipient in the therapy of DED. Ocular compatibility tests were performed to determine its applicability as a potential excipient in eye drops.

2. Materials and Methods

2.1. Materials. For the synthesis of the polymers, L-aspartic acid (Merck, extra pure), phosphoric acid (Sigma Aldrich, 99%), cysteamine (Acros Organics, 95%), *N*,*N*-dimethylethylenediamine (Sigma Aldrich, 95%), ethyl acetate (Reanal Hungary, a.r.), acetone (Reanal Hungary, a.r.), and *N*,*N*-dimethylformamide (DMF) were used without further purification. To mimic the oxidative effect on the ocular surface, 20% w/w 1 M $NaBrO_3$ was used as model oxidant in the formulations. A phosphate-buffered saline (PBS) solution of pH = 7.4 was prepared by dissolving 8 g dm^{-3} NaCl, 0.2 g dm^{-3} KCl, 1.44 g dm^{-3} $Na_2HPO_4{\cdot}2H_2O$, and 0.12 g dm^{-3} KH_2PO_4 in distilled water, with the pH being adjusted with 0.1 M HCl. Lacrimal fluid of pH = 7.4 was prepared by dissolving 2.2 g dm^{-3} $NaHCO_3$, 6.26 g dm^{-3} NaCl, 1.79 g dm^{-3} KCl, 96.4 mg dm^{-} $MgCl_2{\cdot}6H_2O$, and 73.5 mg dm^{-3} $CaCl_2{\cdot}H_2O$ in distilled water, with the pH being adjusted with 1 M HCl. Mucin (porcine gastric mucin type II) was purchased from Sigma Aldrich. Mucin dispersions were prepared with simulated lacrimal fluid and stirred for 8 h. As reference system eye drop formulation from the market was used, consisting of dextran, hypromellose, benzalkonium chloride, EDTA, KCl, NaCl, and water for injection, with the pH being adjusted with HCl and NaOH. Sodium hyaluronate (HA) (MW: 4350 kDa) was purchased from RichterGedeon Ltd. (Budapest, Hungary).

2.2. Synthesis of Cationic ThioPASP-DME Polymers. The precursor polymer of cationic ThioPASP, polysuccinimide (PSI), was synthesized by the thermal polycondensation of L-aspartic acid in a solvent-free reaction at high temperature and reduced pressure. PSI and cysteamine were dissolved in DMF under a nitrogen atmosphere and the solution was stirred for 72 h at room temperature. An excess of *N*,*N*-dimethylethylenediamine was then added and the mixture was stirred for another 24 h under a nitrogen atmosphere. The polymer was precipitated in an excess of ethyl acetate and washed with ethyl acetate and acetone to yield the free base of poly[(*N*-mercaptoethylaspartamide)-co-(*N*-(*N*′,*N*′-dimethylaminoethyl)aspartamide)] (ThioPASP-DME). The polymers are abbreviated as ThioPASP-DME *X*, where *X* is the percentage molar ratio of the *N*-mercaptoethyl aspartamide to the total number of repeating units (Figure 1).

2.3. Ocular Compatibility Tests. Osmolality and pH were measured in 10% w/w aqueous solutions of ThioPASP-DME. Osmolality measurements based on the freezing point depression of a solution were carried out with an automatic osmometer (Knauer Semimicro Osmometer, Germany) in 3 parallels. 150 μL of the solution in a test tube was placed into the instrument, and the sample was overcooled to a temperature lower than its freezing point. Mixing was next applied, which promoted crystallization of the sample. During the crystallization, the temperature automatically rose to the freezing point of the sample and remained at that

temperature for a time. The osmolality (in mOsmol L^{-1}) of the sample was calculated from the freezing point depression.

The pH of ThioPASP-DME solutions prepared with distilled water was determined with a pH meter (Testo 206-pH2, UK) [10].

2.4. Optical Tests. Optical tests were performed by the measurement of transmittance with a UV-spectrophotometer (Thermo Scientific Evolution 201 UV-Visible Spectrophotometer, Thermo Fischer Scientific, Shanghai, China) in the wavelength range 200–800 nm. In our investigations, the thickness of the samples was 10 mm. The transmittance in aqueous solutions of ThioPASP-DME was determined at 10% w/w.

The *refractive index* of the same solution was measured with an Abbe refractometer [10].

2.5. Wettability of Ocular Surfaces. The wettability of ocular surfaces with cationic ThioPASP formulations (10% w/w ThioPASP-DME polymers in PBS) was studied with an OCA Contact Angle System (Dataphysics OCA 20, Dataphysics Inc., GmbH, Germany). Microscopic slides were covered with 20 μL cm^{-2} 5% w/w mucin dispersion in PBS and dried at room temperature for 24 h to model the ocular surface. Drops of ThioPASP-DME solutions were deposited on the surfaces. The degree of wetting was determined by measuring the contact angle by drop shape analysis. If the contact angle of the drops is <90°, the applied system will probably spread easily on the ocular surface, which can promote the interactions between the mucus layer and the formulation.

2.6. Rheology. The effect of the oxidative agent on the polymer solutions and the interaction between the polymer solution and the ocular mucin were investigated by rheology. The rheological properties were studied with a Physica MCR101 rheometer (Anton Paar, Austria). The measuring device was cone and plate type (the diameter was 25 mm, the gap height in the middle of the cone was 0.046 mm, and the cone angle was 1°). ThioPASP-DME was dissolved in PBS and the gelation test was initiated by the addition of model oxidant. For the investigation of the interaction between the polymer and the ocular mucin, the polymer was mixed with a mucin dispersion in PBS and in the presence of 20% w/w model oxidant (the final mucin concentration was 5% w/w, while the final polymer concentration was 10% w/w). As blank measurement, the polymer solution without mucin was measured. The structural changes in the formulation were characterized by frequency sweep tests. The oxidative effect on the eye can induce gelation, and the interactions between ThioPASP-DME and mucin can also result in structural changes; the storage modulus (G') was therefore measured in two different rheological tests. G' indicates the gel state and can also provide information on the strength of the interactions. The higher the value of G', the stronger the gel structure formed. In the first rheological test, G' was plotted for 20 min after the addition of model oxidant, using a strain of 1% and an angular frequency of 0.1 s^{-1} at 25°C. This test follows the possible gelation process. In the second rheological test, G' was determined over the angular frequency range from 0.1 to 100 s^{-1}, at a strain of 1% and at 25°C. This test provides information concerning the structure and the strength of the interactions [9].

2.7. Tensile Test. Tensile test also provides information on the interfacial interaction of the polymer and the ocular surface. Measurements were performed with a TA-XT Plus (Texture analyser (ENCO, Spinea, I)) instrument equipped with a 1 kg load cell and a cylinder probe with a diameter of 1 cm. The force and work needed to separate the polymer solution from the ocular surface are measured, which can characterize the strength of the interaction. Three different test conditions were used: the ocular surface was modelled (1) with 50 μL of an 8% w/w mucin dispersion made with simulated lacrimal fluid (pH = 7.4) on a filter paper (*in vitro* condition), (2) with excised porcine conjunctiva (*ex vivo* condition), and (3) with simulated lacrimal fluid on a filter paper (as a blank measurement).

The porcine conjunctiva was obtained from a slaughterhouse, freshly detached from the connective tissue and stored at −20°C until the measurement. 10 parallel measurements were carried out. Test conditions were as follows: 20 μL of the ThioPASP-DME (containing 20% w/w oxidant and 10% w/w polymer) and HA (0.5 and 1.0% w/w) solutions were attached to a cylinder probe and placed in contact with the test substrates (*in vitro*, *ex vivo*, and blank). A 2500 mN preload was used for 3 min to establish intimate contact between the sample and the test surface. The cylinder probe was then moved upwards to separate the sample from the substrate at a prefixed speed of 2.5 mm min^{-1}. The work of adhesion (A, mN·mm) was calculated as the area under the force versus displacement curve (AUC) [9].

2.8. Statistical Analysis. The results were evaluated and analysed statistically with GraphPad Prism software (version 5). One-way and two-way ANOVA (with Bonferroni posttests) analysis were applied [11]. The values are expressed as means ± standard deviation (SD). A level of $p \leq 0.05$ was taken as significant, $p \leq 0.01$ as very significant, and $p \leq 0.001$ as highly significant.

3. Results

3.1. Ocular Compatibility Tests. During ocular drug delivery formulation, several excipients are used which can change the physical and physiological properties of the ocular surface and the stability of the tear film [4, 12, 13]. The osmolality and the pH of the ThioPASP-DME solutions were therefore measured to determine the physicochemical properties of the solutions. The results are presented in Table 1.

Aqueous solutions of ThioPASP-DME polymers showed strong hypoosmolality (<100 mOsmol L^{-1}), while the reference system was close to isotonic (301.4 mOsmol L^{-1}). The solutions were alkaline (pH > 7). In order to modify the pH of the polymer solution close to that of the tear film (pH = 7.4), the synthesis was extended with a neutralization step. As a result, the pH of this polymer solution was approximately the

Table 1: Osmolality and pH of aqueous ThioPASP-DME solutions (10% w/w).

ThioPASP-DME degree of modification (% *n*/*n*)	Osmolality (mOsm/L) in water Mean ± SD	pH
10	87 ± 0	8.79
10*	183.67 ± 1.25	6.07
20	90 ± 2.94	8.80
30	89.67 ± 0.47	8.77
Reference	282 ± 2.45	6.65

*Neutralized with HCl.

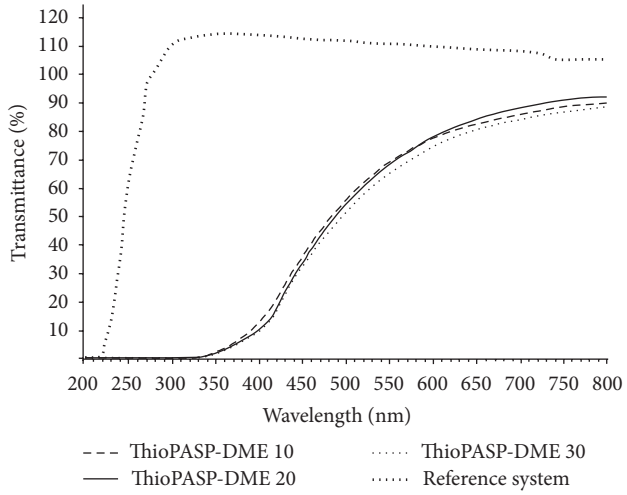

Figure 2: Transmittance of ThioPASP-DME solutions.

physiological pH and that of the reference system (pH = 6.07), while the osmolality increased but remained hypoosmotic (<200 mOsmol L^{-1}).

3.2. Optical Tests. Transmittance spectra of 10% w/w ThioPASP-DME solutions were determined to study the effects of the solutions on the vision. The transmittance curves are depicted in Figure 2.

The ThioPASP-DME solutions are not colourless but slightly yellow, though the transmittance is high over almost the whole range of the visible spectrum. There was no significant effect of the degree of modification (composition) of the ThioPASP-DME. Interestingly, the polymer solutions exhibited a noteworthy UV cut-off at 350 nm; this behaviour can be favourable in the event of eyes exposed to heavy UV radiation.

The refractive indices of the ThioPASP–DME 10, 20, and 30 and the reference solutions were 1.3483, 1.3491, 1.3499, and 1.3350, respectively.

3.3. Wettability of the Ocular Surface. As it is intended to use the ThioPASP-DME solutions in liquid eye drops, their spreading on the ocular surface is an important aspect. In our tests, the ocular surface was modelled with a microscope slide covered with a mucin dispersion. The measured contact angles are to be seen in Figure 3.

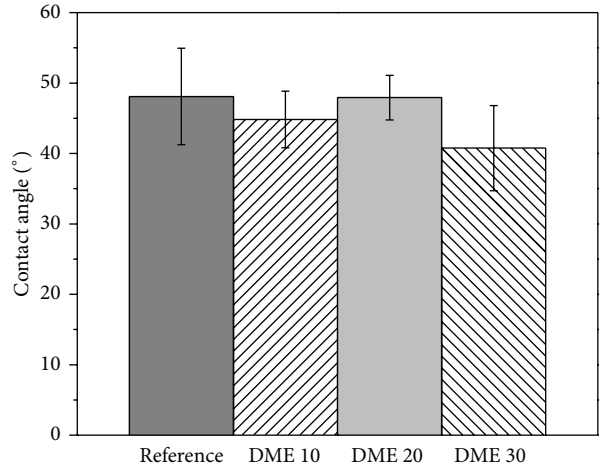

Figure 3: Contact angles of ThioPASP-DME solutions.

The results indicate that the tested polymer compositions provide favourable wetting conditions on the model surface, because the contact angle is <90°.

3.3.1. Rheology. The ThioPASP polymer solutions exhibited *in situ* gelling [9]; the gelation ability of ThioPASP-DME solutions was also tested. In the *in vitro* tests, *in vivo* factors that affected the gelling properties were applied, such as the model oxidant (as oxidative stress) and mucin (as a physiological component of tear film). The gelation (storage modulus (G')) was first determined with and without mucin in the presence of the oxidant.

No gelation was observed in the case of ThioPASP-DME solutions. The G' values did not increase during the examination time, which was in contrast with findings in our previous work, in which solutions of ThioPASP demonstrated abrupt increases in G' within a few minutes. Even the addition of mucin did not induce gelation in the case of ThioPASP-DME.

Frequency sweep tests were performed with the aim of determining any synergetic interaction between the ThioPASP-DME and the mucin (Figure 4). This method is based on the determination of synergistic increases in rheological parameters (G') after the sample is mixed with a mucin dispersion. The increase in G' is caused by chemical and physical bond formation between the mucin and the polymer chains [9, 14–16].

A minor increase in G' was observed in the presence of mucin for the lower degrees of modification, indicating the interaction of the polymer and the mucin. The modulus depended strongly on the angular frequency for the same compositions without mucin and the frequency dependence was slightly reduced in the presence of mucin, suggesting the formation of a weak network. These differences were not observed for the highest degree of modification (ThioPASP-DME 30), where a rather frequency-independent G' was observed both with and without mucin.

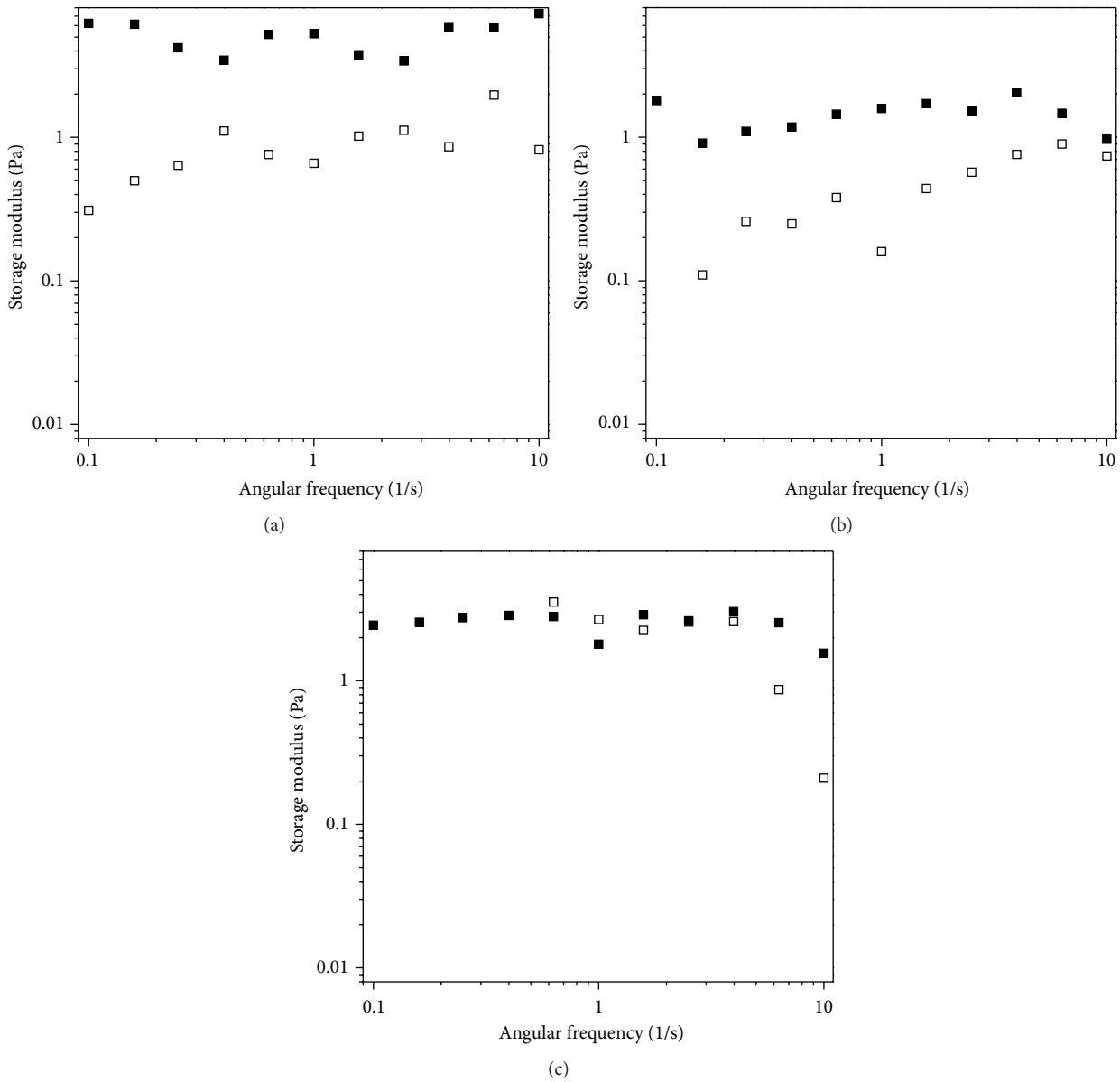

Figure 4: Frequency sweep tests of (a) ThioPASP-DME 10, (b) ThioPASP-DME 20, and (c) ThioPASP-DME 30 with (filled symbols) or without (open symbols) mucin.

3.3.2. Tensile Test. Force was measured as a function of displacement during tensile tests. The adhesive force (the maximum in the curve) and the work of adhesion (the AUC) were calculated [17]. The possible adhesion of ThioPASP-DME solutions to the ocular surface was determined through contacts with lacrimal fluid (blank), mucin dispersion (*in vitro*), and porcine eye conjunctiva (*ex vivo*). The adhesive force (F) and the work of adhesion (A) are shown in Figure 5.

Comparison of the blank with the *in vitro* and *ex vivo* results revealed significant increases in F and A (Figure 5), reflecting the interactions of the ThioPASP-DME polymer with the model surfaces. The highest values were observed in the case of the excised porcine conjunctiva, suggesting that the polymer interacts not merely with the mucin, but also with the other components of the ocular surface. The adhesive force and the work of adhesion values did not vary appreciably with the composition, but the substrate applied during the measurements affected these values strongly, as discussed below.

The mucoadhesivity of the new polymers was compared with that of hyaluronic acid solutions. HA as viscosity enhancing agent has been investigated for years as an active component of formulations applied in DED. Sodium hyaluronate increases the residence time and the precorneal tear film stability and the corneal wettability. It also decreases the evaporation rate of the tear film and improves the healing mechanisms of the cornea [18–21]. The generally applied concentration of HA in artificial tear is 0.1–0.5% w/w. In our

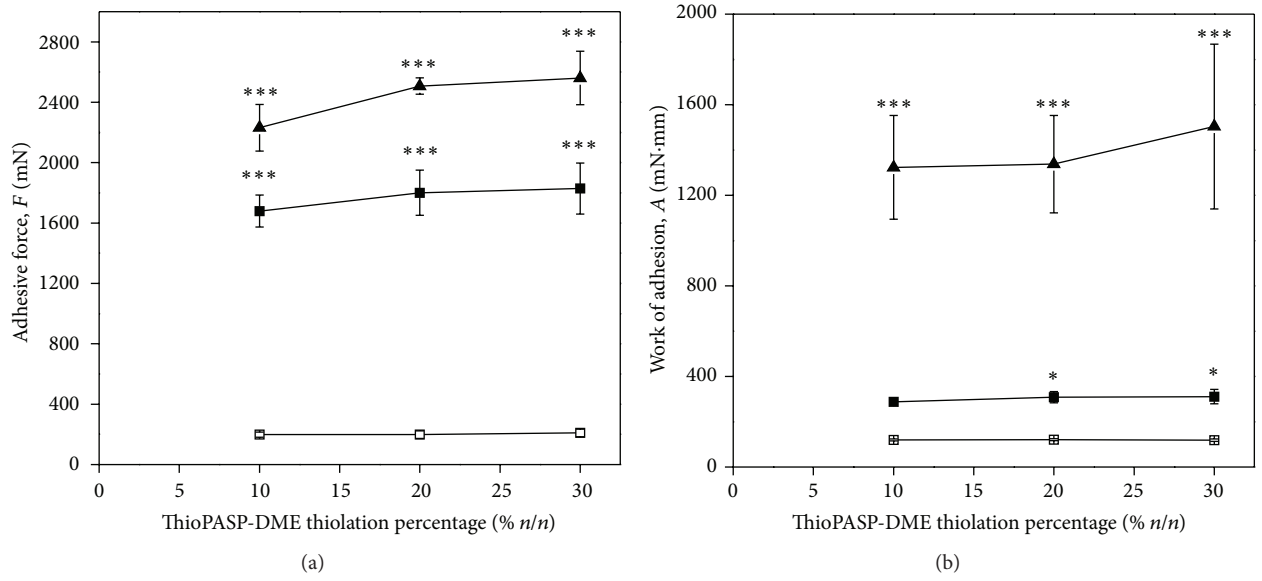

Figure 5: (a) Adhesive force and (b) work of adhesion of ThioPASP-DME solutions under (□) blank, (■) *in vitro*, and (▲) *ex vivo* conditions as functions of the polymer modification ($^{*}P \leq 0.05$, significant difference from the blank; and $^{***}P \leq 0.001$, highly significant difference from the blank).

work, mucoadhesion of 0.5 and 1.0% w/w HA solutions on porcine conjunctiva was measured and compared with that of 10% ThioPASP-DME 10 solution.

Under *ex vivo* condition, the ThioPASP-DME 10 displayed significantly higher mucoadhesivity compared with that of HAs (Figure 6). This phenomenon can be explained by the structure of the new cationic polymer. The elevated work of adhesion value may indicate the formation of disulfide bond and ionic interactions between the polymer chains and the ocular surface, while the viscosity of the polymer solution remained at a moderate level. Complex viscosity of the 0.5% HA, 1.0% w/w HA, and ThioPASP-DME solutions (at 10 Hz) were 80, 1680, and 580 mPas, respectively. Increase of the HA concentration from 0.5 to 1.0 did not affect the work of adhesion.

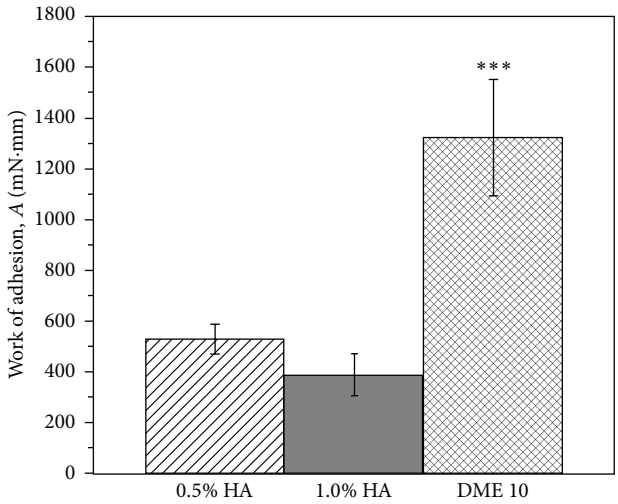

Figure 6: Work of adhesion of polymer solutions under *ex vivo* conditions ($^{***}P \leq 0.001$, highly significant difference from 0.5% and 1.0% w/w HA).

4. Discussion

DED is a multifunctional disease involving the tears and the ocular surface, associated with an increased osmolality of the tear film and inflammation of the ocular surface. The two most common causes of DED are insufficient tear production and excessive tear evaporation, both of which lead to hyperosmolality, ocular damage, or discomfort [3, 22]. Environmental factors (such as air dryness, pollution, or working close to a computer monitor) may increase a tear film dysfunction and cause further evaporative dry eye [23].

Because of the multifactorial pathology of DED, the therapy tends to be very varied. In the main treatments, artificial tears are used, especially preservative-free products, but unfortunately these provide only palliative therapy. In the event of inflammation, artificial tears are combined with oral omega-3 supplements, mucin secretagogues, short-term steroids, and daily cyclosporine A. When the DED is more severe, autologous serum, oral tetracyclines, prosthetic lenses, and systemic immune-suppressants are administered [2, 3]. Locally applied eye drops are used several times per day, which can cause toxic side effects because of the preservative (especially benzalkonium chloride) present in the formulations. These preservatives are cytotoxic to the ocular surface by modifying the lipid phase of the tear film [19].

Osmolality has been deeply investigated in DED and is considered to be a very important factor. The osmolality of the tears in a normal eye is 310 to 334 mOsm L^{-1}, but in DED the osmolality is higher. One aim of artificial tears is to

counter this hyperosmolality, but the effect is generally only temporary. The osmolality of artificial tears is usually in the interval from 181 to 354 mOsmol L^{-1} [24, 25].

In the treatment of DED, stabilization of the tear film is also very important. The tear film is stable for only a short time, because it ruptures in consequence of the concentration gradients and dispersion forces on the mucus layer. The rupture results in the loss of moisturization of the cornea, so that dry spots are formed, which irritate the corneal nerve endings and induce blinking. Thanks to the eyelid movements, a new tear film spreads over the eye surface. The dispersion forces, the interfacial tension, and the viscous resistance of the mucus layer affect the duration of rupture of the mucin layer and the breakup time of the tear film [4].

When all of these factors are taken into consideration, it appears clear that most of the physicochemical properties of the optimum eye drop formulation must be similar to those of the tear film and it must be hypoosmotic to balance the hyperosmotic tears in DED.

In this work, we synthetized and characterized ThioPASP-DME, cationic thiolated polyaspartamide bearing both cationic tertiary amine and redox-responsive thiol pendant groups, as a potentially mucoadhesive and tear film-stabilizing excipient in the therapy of DED. The aim was the synthesis of a mucin analogue polymer which can interact with the ocular mucin via disulphide linkages and the ionic interactions between the positively charged polymer and the negatively charged mucosal surface. Thanks to these complex interactions, a continuous polymer network is formed on the surface, thereby preserving the tear film with maintenance of the hydration of the ocular surface. We assume that ThioPASP-DME polymers can function as ophthalmic drug demulcents, defined in US Food and Drug Administration (FDA) monograph 21 CFR 349 as water-soluble polymers applied topically to protect and lubricate mucous membrane surfaces and to temper dryness and irritation.

We first investigated the physiological acceptability of our formulations. Eye lubricants are recommended to be neutral or slightly alkaline. The pH of the ThioPASP-DME polymer solutions (pH = 8.7–8.8) was higher than that of normal tears (pH = 7.4) and could be therefore adjusted by using hydrochloric acid.

The polymer solutions (10% w/w) were hypoosmotic (87–90 mOsmol L^{-1}) allowing the addition of other components, which is favourable in the therapy of DED. The neutralization process resulted in lower pH but higher osmolality (183.67 ± 1.25 mOsmol L^{-1}), which is in the range of the osmolality of artificial tears (from 181 to 354 mOsmol L^{-1}) [24, 25], but this also allows the inclusion of further additives to the formulation. Ocular lubricants utilized in DED usually contain electrolytes (e.g., bicarbonate, potassium, and other electrolytes), surfactants, and various types of viscosity-increasing agents [26, 27].

Optical tests were performed in order to determine the degree of visual disturbance caused by these polymer solutions. The transmittance of the polymer solutions is slightly modified over a broad range of the visible spectrum and their refractive indices approximate to that of the tears. Thus, they do not greatly affect the quality of vision, while in addition they have a partial UV-filtering effect, which can be favourable in ophthalmic therapy.

The polymer solutions can readily spread on the simulated eye surface, as indicated by the low contact angles. This means that the formulations have the ability to establish strong interactions with the surface and to resist elimination immediately after administration.

The ThioPASP polymers are redox-sensitive and undergo gelling in response to oxidative stress or agents [9]. The present work revealed that the solutions of the ThioPASP-DME polymers did not form gels in response to an oxidative effect. This behaviour can be advantageous, because a sticky feeling and a foreign body sensation can be avoided and the swelling gel does not cause dehydration. On the other hand, ThioPASP-DME interacts with mucin, as indicated by the elevated G' in rheological experiments, with the polymer therefore remaining on the surface without causing a noteworthy increase in viscosity.

Tensile tests likewise verified the good adhesion of the polymer solution to the ocular surface. Besides hydrogen bonds, thiolated polymers are able to form covalent bonds with the cysteine-rich subdomains of mucin. We additionally immobilized other side groups with cationic, positively charged groups, so that ionic interactions can also occur [4]. Changes in the degree of thiolation did not affect the adhesion appreciably, but an increased degree of thiolation is not recommended because a higher number of thiol side groups may result in lower stability of the polymers against atmospheric oxidation during storage. Oxidation during storage may lead to a lower dissolution rate prior to application. The strongest adhesion was measured on excised porcine conjunctiva, which suggests that not only do the mucin-polymer interactions (disulphide bonds) play a role in the adherence, but other secondary interactions may also develop, improving the efficacy of the formulation.

ThioPASP-DME polymers showed better mucoadhesion compared with conventionally used HAs in DED, while the viscosity of their solution was not elevated.

5. Conclusion

We successfully adjusted the properties of ThioPASP-DME (pH and osmolality) to the desired physiological levels thereby resulting in a possibility to decrease side effects such as irritation and dehydration. In consequence of their similar structure to that of mucin, ThioPASP-DME solutions also have the ability to stabilize the tear film. They can interact with the ocular mucin and provide strong adhesion, ensuring an improved residence time and prolonged hydration of the ocular surface. Further beneficial properties of the polymer solutions, such as good spreading on the ocular surface, marked transmittance, and a partial UV-filtering effect, can provide new possibilities in the therapy of DED.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgments

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References

[1] H. Lorentz and H. Sheardown, "Ocular delivery of biopharmaceuticals," in *Mucosal Delivery of Biopharmaceuticals Biology, Challenges and Strategies*, J. Neves and B. Sarmento, Eds., pp. 221–259, Springer, New York, NY, USA, 2014.

[2] H. Brewitt and F. Sistani, "Dry eye disease: the scale of the problem," *Survey of Ophthalmology*, vol. 45, supplement 2, pp. S199–S202, 2001.

[3] H. Lin and S. C. Yiu, "Dry eye disease: a review of diagnostic approaches and treatments," *Saudi Journal of Ophthalmology*, vol. 28, no. 3, pp. 173–181, 2014.

[4] A. Ludwig, "The use of mucoadhesive polymers in ocular drug delivery," *Advanced Drug Delivery Reviews*, vol. 57, no. 11, pp. 1595–1639, 2005.

[5] P. Argüeso and I. K. Gipson, "Epithelial mucins of the ocular surface: structure, biosynthesis and function," *Experimental Eye Research*, vol. 73, no. 3, pp. 281–289, 2001.

[6] S. Bonengel and A. Bernkop-Schnürch, "Thiomers—from bench to market," *Journal of Controlled Release*, vol. 195, pp. 120–129, 2014.

[7] S. M. Thombre and B. D. Sarwade, "Synthesis and biodegradability of polyaspartic acid: a critical review," *Journal of Macromolecular Science—Part A*, vol. 42, no. 9, pp. 1299–1315, 2005.

[8] S. Roweton, S. J. Huang, and G. Swift, "Poly(aspartic acid): synthesis, biodegradation, and current applications," *Journal of Environmental Polymer Degradation*, vol. 5, no. 3, pp. 175–181, 1997.

[9] G. Horvát, B. Gyarmati, S. Berkó et al., "Thiolated poly(aspartic acid) as potential in situ gelling, ocular mucoadhesive drug delivery system," *European Journal of Pharmaceutical Sciences*, vol. 67, pp. 1–11, 2015.

[10] M. Budai-Szűcs, G. Horvát, B. Gyarmati et al., "*In vitro* testing of thiolated poly(aspartic acid) from ophthalmic formulation aspects," *Drug Development and Industrial Pharmacy*, vol. 42, no. 8, pp. 1241–1246, 2016.

[11] J. Patterson, R. Siew, S. W. Herring, A. S. P. Lin, R. Guldberg, and P. S. Stayton, "Hyaluronic acid hydrogels with controlled degradation properties for oriented bone regeneration," *Biomaterials*, vol. 31, no. 26, pp. 6772–6781, 2010.

[12] S. Patel, K. E. Boyd, and J. Burns, "Age, stability of the precorneal tear film and the refractive index of tears," *Contact Lens and Anterior Eye*, vol. 23, no. 2, pp. 44–47, 2000.

[13] B. Yañez-Soto, M. J. Mannis, I. R. Schwab et al., "Interfacial phenomena and the ocular surface," *Ocular Surface*, vol. 12, no. 3, pp. 178–201, 2014.

[14] C. M. Caramella, S. Rossi, and M. C. Bonferoni, "A rheological approach to explain the mucoadhesive behavior of polymer hydrogels," in *Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development*, E. Mathiowitz, D. E. Chickering III, and C. M. Lehr, Eds., pp. 25–65, Marcel Dekker, New York, NY, USA, 1999.

[15] E. E. Hassan and J. M. Gallo, "A simple rheological method for the *in vitro* assessment of mucin-polymer bioadhesive bond strength," *Pharmaceutical Research*, vol. 7, no. 5, pp. 491–495, 1990.

[16] F. Madsen, K. Eberth, and J. D. Smart, "A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration," *Journal of Controlled Release*, vol. 50, no. 1-3, pp. 167–178, 1998.

[17] C. Woertz, M. Preis, J. Breitkreutz, and P. Kleinebudde, "Assessment of test methods evaluating mucoadhesive polymers and dosage forms: an overview," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 85, no. 3, pp. 843–853, 2013.

[18] B. B. Sand, K. Marner, and M. S. Norn, "Sodium hyaluronate in the treatment of keratoconjunctivitis sicca. A double masked clinical trial," *Acta Ophthalmologica*, vol. 67, no. 2, pp. 181–183, 1989.

[19] V. Papa, P. Aragona, S. Russo, A. Di Bella, P. Russo, and G. Milazzo, "Comparison of hypotonic and isotonic solutions containing sodium hyaluronate on the symptomatic treatment of dry eye patients," *Ophthalmologica*, vol. 215, no. 2, pp. 124–127, 2001.

[20] T. Hamano, K. Horimoto, M. Lee, and S. Komemushi, "Sodium hyaluronate eyedrops enhance tear film stability," *Japanese Journal of Ophthalmology*, vol. 40, no. 1, pp. 62–65, 1996.

[21] J. D. Nelson and R. L. Farris, "Sodium hyaluronate and polyvinyl alcohol artificial tear preparations. A comparison in patients with keratoconjunctivitis sicca," *Archives of Ophthalmology*, vol. 106, no. 4, pp. 484–487, 1988.

[22] G. N. Foulks, "The correlation between the tear film lipid layer and dry eye disease," *Survey of Ophthalmology*, vol. 52, no. 4, pp. 369–374, 2007.

[23] C. Baudouin, "The pathology of dry eye," *Survey of Ophthalmology*, vol. 45, supplement 2, pp. S211–S220, 2001.

[24] "Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye Work Shop," *The Ocular Surface*, vol. 5, no. 2, pp. 163–178, 2007.

[25] P. Wright, M. Cooper, and A. M. Gilvarry, "Effect of osmolarity of artificial tear drops on relief of dry eye symptoms: BJ6 and beyond," *British Journal of Ophthalmology*, vol. 71, no. 2, pp. 161–164, 1987.

[26] J. P. Gilbard, S. R. Rossi, and K. G. Heyda, "Ophthalmic solutions, the ocular surface, and a unique therapeutic artificial tear formulation," *American Journal of Ophthalmology*, vol. 107, no. 4, pp. 348–355, 1989.

[27] K. Green, D. L. MacKeen, T. Slagle, and L. Cheeks, "Tear potassium contributes to maintenance of corneal thickness," *Ophthalmic Research*, vol. 24, no. 2, pp. 99–102, 1992.

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Research Article

Diagnostic Performance of McMonnies Questionnaire as a Screening Survey for Dry Eye: A Multicenter Analysis

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Purpose. To evaluate the diagnostic performance of the McMonnies questionnaire as a screening survey for dry eye in Chinese outpatients. *Methods*. The questionnaire was self-administered by 27,999 patients with dry eye symptoms. A thorough ophthalmic examination including tear break-up time (TBUT), fluorescein staining, and Schirmer I test was completed to make a clinical diagnosis of dry eye. Reliability, validity, and accuracy of the McMonnies questionnaire were assessed. *Results*. The McMonnies questionnaire showed poor internal consistency (Cronbach $\alpha = 0.37$), but excellent validity as the scores correlated with TBUT (Spearman test, $r = -0.322$, $P < 0.001$) and Schirmer I test (Spearman's test, $r = -0.370$, $P < 0.001$), and significantly differed between the dry eye and control groups (2-sample t-test, $t = 69.51$, $P < 0.001$). The area under the receiver-operating characteristics (ROC) curve (AUC) was 0.729, suggesting moderate accuracy in identifying dry eye and non-dry eye patients. However, the AUCs varied significantly in different gender and age subgroups (z test, $P < 0.001$), as the discriminating ability declined with age. Analysis of the ROC curves also revealed that different cut-off points should be employed for each subgroup to achieve the same level of accuracy. *Conclusions*. The McMonnies questionnaire demonstrates moderate diagnostic value, and different cut-off points should be selected for various study populations.

1. Introduction

Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface [1]. Dry eye could be either a separated clinical entity or a condition associated with other systemic or ocular surface diseases. Currently, it belongs to the most frequently encountered ocular problems in clinical practice.

As the definition of dry eye is still under constant revision, the lack of a "gold standard" for diagnosis challenges ophthalmologists worldwide. The International Dry Eye Workshop recommended confirming the diagnosis of dry eye based on a combination of symptoms and objective clinical tests [2]. Accordingly, the Chinese diagnostic criteria of dry eye were published by the Corneal Disease Study Group of Chinese Ophthalmological Society in 2013, offering a more defined standard [3].

The subjective symptoms of dry eye, along with certain risk factors, were advised to be screened by validated questionnaires [2]. As one of the most long-standing instruments, the McMonnies questionnaire is widely used in numerous prevalence studies [4–6] and clinical trials [7–9]. It contains 14 questions that revolve around the risk factors of dry eye, including age, gender, previous dry eye treatments, dry eye-related symptoms (both primary and secondary to environmental triggers), and systemic conditions associated with dry eye (dryness of mucous membranes, arthritis, thyroid disease, and medication use) [10].

Previous analyses [11–14] revealed varying values of sensitivity (34%–98%) and specificity (36%–97%) for the McMonnies questionnaire. Moreover, this instrument was originally devised from a sample of Australian women aged above 45 years with or without keratoconjunctivitis sicca syndrome [10, 11]. Subsequent studies [12–14] evaluating the reliability and validity of the instrument continued to focus on non-Asian populations. Thus, it is anticipated that variations in the diagnostic efficacy are likely to occur when applying the instrument to a Chinese cohort.

Therefore, we carried out a study in multiple ophthalmological centers across China to investigate the diagnostic performance of the McMonnies questionnaire as a screening survey for dry eye in Chinese outpatients.

2. Method

2.1. Patient Sample. The study was carried out in 94 ophthalmological centers, distributed in 45 cities, 23 provinces across China. Consecutive outpatients in general eye clinics were enrolled from July to November, 2013, if they presented with one or more of the following chief complaints: dryness, grittiness, burning sensation, tiredness, soreness, and visual disturbance. Participants are excluded if they exhibited any active infection of the eye, evidence of ocular chemical or thermal burn, ocular surgeries within 6 months before the screening, and pregnancy or lactation. Informed consents were obtained for each patient at each clinical site. The research was approved by Peking University Third Hospital Medical Ethics Committee and consistent with the tenets of the Declaration of Helsinki.

2.2. Assessment of Dry Eye. The McMonnies questionnaire, translated into Mandarin in advance, was self-administered by all participants. Then, each patient would be assessed by an ophthalmologist. The examining doctor had no knowledge of the results of the completed questionnaires. All subjects were required to remove their contact lens and discontinue any artificial tears for at least 2 hours before the assessment. Ophthalmic examinations were conducted in the following order:

(1) *Inquiry of Medical History*. Information on ophthalmic and systemic disease was collected.

(2) *Tear Break-Up Time (TBUT)*. A standard fluorescein stripe was moistened and used to lightly touch the inferior palpebral conjunctiva. The patient would be asked to blink several times. Under cobalt blue light of a slit-lamp, the time interval between the last blink and the appearance of the first desiccation spot would be recorded as TBUT.

(3) *Keratoconjunctival Staining*. After TBUT test, any fluorescein staining of the corneas and interpalpebral conjunctiva was also recorded.

(4) *Schirmer I Test*. Without anesthesia, a precalibrated standard stripe was placed in the lateral one-third of each lower fornix for 5 minutes. During this time, the patients were instructed to look downward or gently close their eyes. The length of the wetting was measured after removing the stripe.

(5) *Slit-Lamp Exam*. Eyelid margins, including meibomian gland orifices and secretions, were evaluated under slit-lamp for pathological changes.

Test results of the more severely affected eye were recorded for further analyses. Diagnoses were established according to the Chinese diagnostic criteria of dry eye [3]: (1) presence of dry eye symptoms (dryness, grittiness, burning sensation, tiredness, soreness, or visual disturbance), with TBUT $\leq$ 5 s or Schirmer I test $\leq$ 5 mm/5 min; (2) presence of dry eye symptoms, with 5 s < TBUT $\leq$ 10 s or 5 mm/5 min < Schirmer I test $\leq$ 10 mm/5 min, accompanied by positive keratoconjunctival staining with fluorescein. Subjects conformed to either of the two criteria were clinically diagnosed with dry eye; otherwise they were classified as non-dry eye (control).

2.3. Statistical Analyses. Data analyses were performed using Statistical Package for the Social Sciences software, version 22.0 (SPSS Inc., Chicago, IL). A P value less than 0.05 was considered statistically significant, and 95% confidence intervals (CIs) were tabulated.

2.3.1. Factor Analysis. The Kaiser-Meyer-Olkin measure was first calculated to test the degree of common variance, an assessment of whether the sample is adequate for factor analysis. Any value below 0.50 is interpreted as "unacceptable" for factor analysis. The Bartlett test of sphericity was also conducted to determine whether the items were sufficiently intercorrelated for factor analysis.

After weighing the adequacy, factor analysis with varimax rotation was performed to find out whether the items of the McMonnies questionnaire tend to cluster into certain domains.

2.3.2. Reliability. The internal reliability of the McMonnies questionnaire was evaluated by Cronbach α. Generally, an α value greater than 0.70 is acceptable to indicate that the items of the instrument are measuring the same thing. It should be noted that the Cronbach α coefficient is an index dependent on the number of items in an instrument. Therefore, the average interitem correlation was also calculated, which is not affected by the number of items.

Due to the large sample size of our study, all participants only completed the questionnaire once. So we were unable to assess the test-retest reliability in this study.

2.3.3. Validity. Concurrent validity was assessed by examining the correlation between scores of the questionnaire and 2 quantitative dry eye test results (i.e., TBUT and the Schirmer I test) using Spearman's rank correlation. Discriminant validity was evaluated using 2-sample t-test to determine the differences in scores between the dry eye and the control group.

2.3.4. Accuracy. In order to maximize the diagnostic efficacy of the McMonnies questionnaire, receiver-operating characteristics (ROC) curves of both the entire sample and different gender and age groups were generated. ROC curves express the diagnostic accuracy of a test variable by plotting the sensitivity of the test against the specificity at all possible thresholds. This method was employed to select the most appropriate cut-off point for our study population.

The area under the ROC curve (AUC) is an index for diagnostic value: 0.5 means no discrimination between the affected and the control groups, while 1.0 indicates perfect discrimination. We compared the ROC curves of different gender and age groups using z tests to see if the diagnostic

Table 1: Demographics of the study population and prevalence of dry eye in each gender/age group.

Gender/age	Sample size	Prevalence (%)
Overall	27999	58.8
Male		
Under 25 years	3433	51.1
25–45 years	5690	52.7
Over 45 years	4596	59.0
Female		
Under 25 years	2793	45.2
25–45 years	6784	62.8
Over 45 years	4703	73.9

performance is compromised when applying the instrument to certain subpopulations.

We also summarized the positive likelihood ratio (LR+) of the McMonnies questionnaire in different groups, which is a measure that indicates how much the odds of the disease increase when a test is positive.

3. Results

3.1. Study Population. This study recruited 31,124 outpatients from the ophthalmology departments of 94 tertiary hospitals, distributed in 45 cities, 23 provinces across China. Among these participants, 27,999 (90.0%) have completed the questionnaire and all the dry eye tests. The demographics of the study population are listed in Table 1. The majority of our sample (44.6%) belonged to the age group of 25–45 years, whereas participants less than 25 years old made up the smallest portion (22.2%). Females accounted for 51.0% (14,280) of the sample. The overall prevalence of dry eye according to the Chinese diagnostic criteria was 58.8% (16,468), with the remaining 41.2% (11,531) classified as the control group. Prevalence of dry eye in each gender/age subgroup is also revealed in Table 1.

3.2. Factor Analysis. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.62, and the Bartlett test of sphericity was significant ($P < 0.001$), indicating sufficiency for factor analysis. Exploratory factor analysis of the McMonnies questionnaire revealed four potential factors, explaining only 47% of the cumulative variance. Such low communalities suggested that the extracted factors were insufficient to represent each item. Moreover, none of the factor loadings seemed to provide any logical meaning. For instance, one factor was composed of the question regarding a history of thyroid abnormality and the question about sleeping with eyes partly open. Therefore, the attempt to further analyze the questionnaire by domain was denied.

3.3. Reliability. The Cronbach α based on standardized items for the McMonnies questionnaire was 0.37, indicating poor internal consistency. Similarly, the average interitem correlation was 0.046 (range, −0.132 to 0.328), implying that each item refers to relatively independent aspects of the instrument's objective (i.e., screening dry eye).

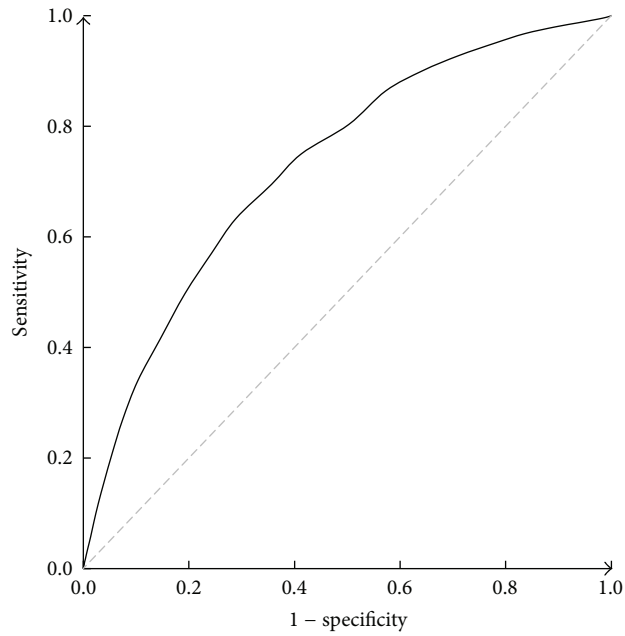

Figure 1: The overall ROC curve of the McMonnies questionnaire for screening dry eye.

3.4. Validity. The correlations between the scores of the McMonnies questionnaire and both dry eye test results were significant. The Spearman coefficient for the questionnaire and TBUT was −0.322 ($P < 0.001$) and −0.370 ($P < 0.001$) for Schirmer I test. Also, the mean scores were 17.27 ± 5.33 for the dry eye group and 12.82 ± 5.21 for the control group, respectively. The scores of the two groups were statistically different from each other (2-sample t-test, $t = 69.51$, $P < 0.001$).

3.5. Accuracy. The ROC curves of the McMonnies questionnaire in the entire sample as well as in various gender/age subgroups are shown in Figures 1 and 2. The sensitivity, specificity, and LR+ values at a cut-off point of 14.5 as recommended [12] are listed in Table 2, along with the AUCs of each group. The overall AUC of McMonnies questionnaire is 0.729 (95% CI, 0.723–0.735), indicating moderate discrimination. All AUCs of each group were significantly different from that of the entire sample (z test, $P < 0.001$), as the discriminating ability declined with age. Likewise, sensitivity, specificity, and LR+ all varied considerably among these cohorts.

Since the McMonnies questionnaire is used mainly as a screening method for dry eye, it is appropriate to maximize the sensitivity value, so as to avoid missed diagnosis. Accordingly, alternative thresholds were introduced for the instrument (Table 3). We also select separate cut-off points for each gender/age subpopulation with the highest specificity after setting the sensitivity at a value above 0.80, that is, those with the sensitivity values most approximate to 0.80 (Table 4).

4. Discussion

To our knowledge, this is the first multicenter study with a large sample investigating the diagnostic efficacy of the

Table 2: Sensitivities, specificities, LR+, and AUCs of the McMonnies questionnaire in different gender/age groups at a recommended cut-off point of 14.5*.

Gender/age	Sensitivity (%)	Specificity (%)	LR+	AUC#
Overall	69.5	64.3	1.95	0.729 (0.723, 0.735)
Male				
Under 25 years	44.3	18.6	0.54	0.752 (0.736, 0.769)
25–45 years	61.7	23.7	0.81	0.734 (0.721, 0.747)
Over 45 years	71.4	47.4	1.36	0.665 (0.649, 0.681)
Female				
Under 25 years	53.3	15.7	0.63	0.767 (0.749, 0.784)
25–45 years	73.6	46.6	1.38	0.699 (0.686, 0.711)
Over 45 years	88.2	69.7	2.91	0.661 (0.643, 0.679)

*LR+: positive likelihood ratio; AUC: area under the receiver-operating characteristics curves.
#Data presented as mean AUC (95% confidence interval).

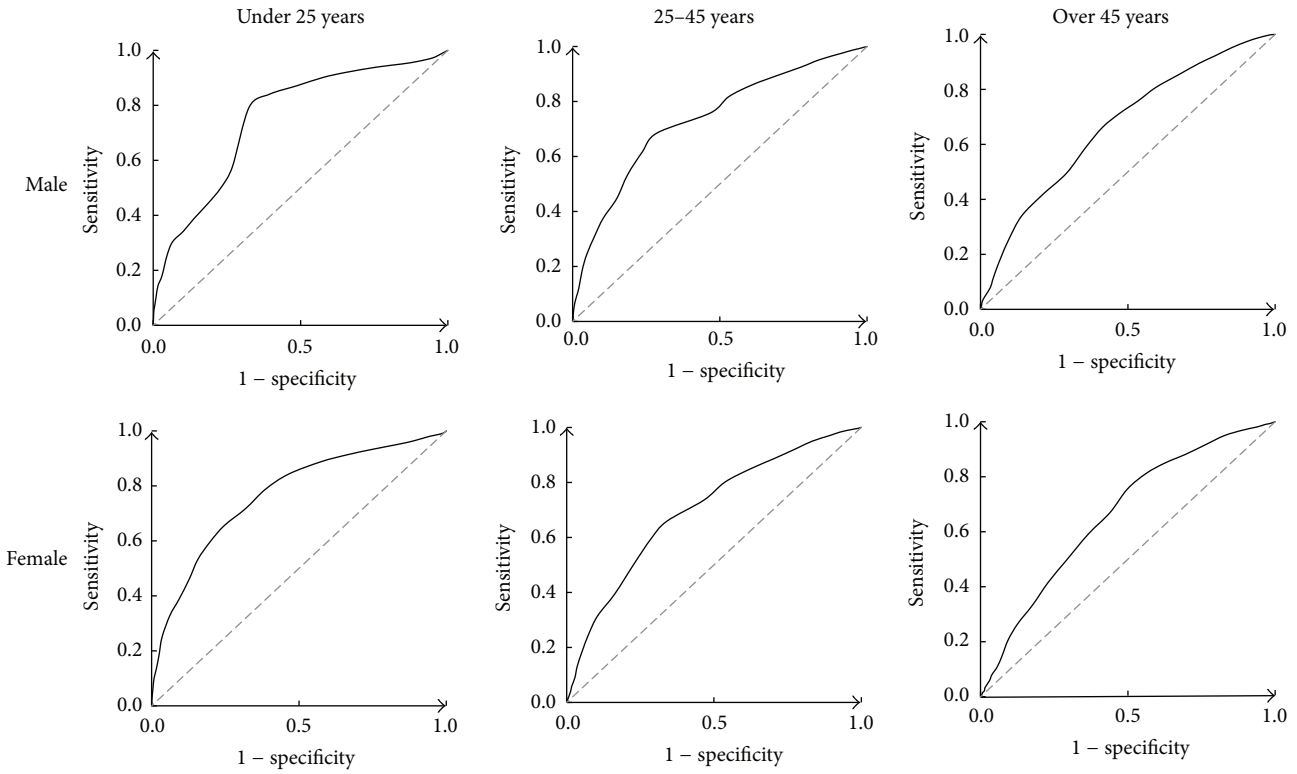

Figure 2: ROC curves of the McMonnies questionnaire in various gender and age groups. Note the relatively rounded curves (towards the upper left corner of the diagram) in younger subgroups, indicating good diagnostic performance.

Table 3: Alternative cut-off points for the McMonnies questionnaire.

Cut-off points	Sensitivity (%)	Specificity (%)	LR+
9.5	92.8	28.9	1.30
10.5	90.0	35.7	1.40
11.5	86.4	42.7	1.51
12.5	80.3	49.7	1.60
13.5	75.0	58.7	1.82
14.5	69.5	64.3	1.95
15.5	63.3	71.1	2.19
16.5	57.0	75.7	2.35

McMonnies questionnaire. Our results suggest that the instrument shows poor internal consistency, excellent validity, and moderate discriminating ability as a screening survey for dry eye in Chinese outpatients.

The McMonnies questionnaire was initially developed by reviewing literature, and scores of each item were tabulated based on clinical experience [10]. Several reports evaluating the diagnostic efficacy of the questionnaire have been published since then. McMonnies and Ho [11] tested the instrument in 100 women aged above 45 years with or without keratoconjunctivitis sicca and achieved 98% sensitivity and 97% specificity. The results were deemed biased, because they

Table 4: Sensitivities, specificities, and LR+ of the McMonnies questionnaire in different gender/age groups at newly proposed cut-off points*.

Gender/age	Proposed cut-off points#	Sensitivity (%)	Specificity (%)	LR+
Overall	12.5	80.3	49.7	1.60
Male				
Under 25 years	10.5	84.0	60.5	2.13
25–45 years	11.5	81.7	47.0	1.54
Over 45 years	12.5	80.7	40.5	1.36
Female				
Under 25 years	9.5	84.2	53.6	1.81
25–45 years	13.5	80.1	46.3	1.49
Over 45 years	16.5	81.2	43.8	1.44

*LR+: positive likelihood ratio.
#Proposed cut-off points of each group are those with the highest specificity after setting the sensitivity at a value above 0.80.

were derived from the same sample from which the cut-off value was determined. So they reassessed on an independent sample of 50 women with Sjögren syndrome and 124 normal controls, all over 45 years of age, and found a sensitivity of 92% and specificity of 93% with a weighted-scale algorithm [12]. Still, the data were affected by spectrum bias, since the severity of the disease in the study population was highly selective. Later on, Nichols et al. [14] reported a sensitivity and specificity of 82% and 36%, respectively, by identifying various degrees of dry eye severity in a sample without normal controls. Moreover, differences in the diagnostic criteria of dry eye used in these studies made it challenging to compare the results.

Nonetheless, certain psychometric properties of the McMonnies questionnaire can be compared with other studies, because they are irrelevant to diagnostic criteria. In our study, the questionnaire showed poor internal consistency as indicated by the Cronbach α and average interitem correlation. Such low internal reliability implies that each item of the instrument measures rather independent aspects of dry eye. This is also why the items failed to cluster into any logical domains by factor analysis. These were consistent with the results of Nichols et al. [14]. The authors inferred that the poor internal consistency would undermine the power associated with statistical significance tests, when comparing the scores between different groups or over time. Similar conclusions have been made using Rasch analysis [15], suggesting that the McMonnies questionnaire does not function as a measure.

Unlike previous studies, the instrument was found to have fine validity in our study population. The scores not only differed significantly between the dry eye and control groups but also strongly correlated with the results of TBUT and Schirmer I test. Several prevalence studies [16, 17] have indicated poor correlations between dry eye symptoms and objective clinical tests. Even so, we would argue that the McMonnies questionnaire is comprised of many aspects of dry eye rather than symptoms. It is possible that the objective test results are correlated with some unknown factors such as age, gender, or secondary symptoms caused by environmental triggers. After all, the Schirmer I test without anesthesia is technically a stimulus to the subjects' eyes. Besides, Hong et al. [18] reported that the Schirmer I test values were correlated with age in a Chinese cohort, while TBUT results were not. Differences in the ethnicity of the study populations may also contribute, which is beyond the scope of our study.

The McMonnies questionnaire showed moderate accuracy in screening dry eye. Further analysis of the ROC curves revealed varying discriminating abilities among different gender and age subgroups, as the AUCs decreased with age. This is a bit surprising, since the instrument was originally developed and adjusted with subjects over 45 years old [11]. Again, variations in experimental samples and criteria used for disease diagnosis should be taken into account here. The differences in diagnostic efficacy among each subgroup are substantial, especially when applied with the same threshold of 14.5 (Table 2). It even resulted with some LR+ values lower than 1.0, which meant that the odds of dry eye actually reduced after the scores of the questionnaire were deemed positive.

Therefore, we believe it is necessary to assign separate cut-off values for different gender and age subpopulation. As a screening method for dry eye, the McMonnies questionnaire is extremely cost-effective as it could be conducted in a self-administered manner by patients. The sensitivity values are suggested to be maximized to avoid missed diagnosis. This is particularly appropriate when the patients could be further assessed with routine ophthalmic examinations to reach a final diagnosis in clinics. Based on these reasons, we consider a sensitivity value over 0.80 acceptable for the screening purpose. The diagnostic performances of each subgroup showed less diversity with newly proposed cut-off points as depicted in Table 4. Nevertheless, we need to stress that these cut-off points were derived from selected Chinese outpatients with at least one of the typical dry eye complaints. Further studies are required to assess these proposed cut-off points for their efficacy on independent samples.

5. Conclusion

Our data suggest that the McMonnies questionnaire demonstrate poor internal consistency, fine validity, and moderate accuracy as a screening survey for dry eye in Chinese outpatients. It is recommend to become a routine process for

dry eye diagnosis in the clinical practice of ophthalmologists. However, different cut-off points should be selected for various subpopulations.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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References

[1] DEWS Definition and Classification Subcommittee, "The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[2] DEWS Diagnostic Methodology Subcommittee, "Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 108–152, 2007.

[3] Corneal Disease Study Group of Chinese Ophthalmological Society, "Expert consensus on clinical diagnosis and treatment of dry eye," *Chinese Journal of Ophthalmology*, vol. 49, no. 1, pp. 73–75, 2013 (Chinese).

[4] J. M. Albietz, "Prevalence of dry eye subtypes in clinical optometry practice," *Optometry and Vision Science*, vol. 77, no. 7, pp. 357–363, 2000.

[5] A. Rege, V. Kulkarni, N. Puthran, and T. Khandgavave, "A clinical study of subtype-based prevalence of dry eye," *Journal of Clinical and Diagnostic Research*, vol. 7, no. 10, pp. 2207–2210, 2013.

[6] L. L. Tan, P. Morgan, Z. Q. Cai, and R. A. Straughan, "Prevalence of and risk factors for symptomatic dry eye disease in Singapore," *Clinical & Experimental Optometry*, vol. 98, no. 1, pp. 45–53, 2015.

[7] M. J. Glasson, F. Stapleton, and M. D. P. Willcox, "Lipid, lipase and lipocalin differences between tolerant and intolerant contact lens wearers," *Current Eye Research*, vol. 25, no. 4, pp. 227–235, 2002.

[8] H. A. Cho, J. J. Cheon, J. S. Lee, S. Y. Kim, and S. S. Chang, "Prevalence of dry eye syndrome after a three-year exposure to a clean room," *Annals of Occupational and Environmental Medicine*, vol. 26, article 26, 2014.

[9] Y. Xu and Y. Yang, "Dry eye after small incision lenticule extraction and LASIK for myopia," *Journal of Refractive Surgery*, vol. 30, no. 3, pp. 186–190, 2014.

[10] C. W. McMonnies, "Key questions in a dry eye history," *Journal of the American Optometric Association*, vol. 57, no. 7, pp. 512–517, 1986.

[11] C. W. McMonnies and A. Ho, "Patient history in screening for dry eye conditions," *Journal of the American Optometric Association*, vol. 58, no. 4, pp. 296–301, 1987.

[12] C. McMonnies, A. Ho, and D. Wakefield, "Optimum dry eye classification using questionnaire responses," *Advances in Experimental Medicine and Biology*, vol. 438, pp. 835–838, 1998.

[13] J. J. Nichols, G. L. Mitchell, K. K. Nichols, R. Chalmers, and C. Begley, "The performance of the contact lens dry eye questionnaire as a screening survey for contact lens-related dry eye," *Cornea*, vol. 21, no. 5, pp. 469–475, 2002.

[14] K. K. Nichols, J. J. Nichols, and G. L. Mitchell, "The reliability and validity of mcmonnies dry eye index," *Cornea*, vol. 23, no. 4, pp. 365–371, 2004.

[15] V. K. Gothwal, K. Pesudovs, T. A. Wright, and C. W. McMonnies, "McMonnies questionnaire: enhancing screening for dry eye syndromes with Rasch analysis," *Investigative Ophthalmology & Visual Science*, vol. 51, no. 3, pp. 1401–1407, 2010.

[16] E. M. Hay, E. Thomas, B. Pal, A. Hajeer, H. Chambers, and A. J. Silman, "Weak association between subjective symptoms of and objective testing for dry eyes and dry mouth: results from a population based study," *Annals of the Rheumatic Diseases*, vol. 57, no. 1, pp. 20–24, 1998.

[17] O. D. Schein, M. C. Hochberg, B. Muñoz et al., "Dry eye and dry mouth in the elderly: a population-based assessment," *Archives of Internal Medicine*, vol. 159, no. 12, pp. 1359–1363, 1999.

[18] J. Hong, Z. Liu, J. Hua et al., "Evaluation of age-related changes in noninvasive tear breakup time," *Optometry and Vision Science*, vol. 91, no. 2, pp. 150–155, 2014.

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Review Article

Dry Eye Syndrome in Patients with Diabetes Mellitus: Prevalence, Etiology, and Clinical Characteristics

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There has been substantial progress in our understanding of the ocular surface system/lacrimal function unit in the past 15 years. Keratoconjunctivitis sicca, more commonly referred to as dry eye syndrome (DES), is the most frequently encountered condition and diabetes mellitus (DM) has been identified as one of the leading causes of DES. Poor glycemic control affects both the anterior and the posterior segments of the eye and increasing prevalence of diabetes-associated DES (DMDES) has been reported in recent years. The pathogenesis and specific features of DMDES remain uncertain and interventions are limited to those used in DES. This review outlines the pathogenesis, clinical manifestations, and the current preventive and treatment strategies for diabetes-related DES.

1. Introduction

The International Diabetes Federation (IDF) estimates that the global diabetes epidemic continues increasing. According to the report of the IDF in 2013, China has the largest number of diabetics (98.4 million) and this number is now higher than in India (65.1 million) and in the USA (24.4 million) [1].

While diabetic retinopathy (DR) and diabetic cataracts are well-known complications, dry eye syndrome (DES), also referred to as keratoconjunctivitis sicca, is also common in the diabetic population. Studies have indicated 54% prevalence of asymptomatic and symptomatic DES, in diabetes [2]. However, the relationship between diabetes and DES still remains unclear. This review aims to discuss the prevalence, etiology, and treatment strategies of diabetes mellitus associated DES and to emphasize the importance of early diagnosis and interventions in diabetes-associated DES.

2. Prevalence of Dry Eye Syndrome in Diabetes Mellitus

Diabetes mellitus (DM) has been identified as one of the leading systemic risk factors for DES. The reported prevalence of DES in diabetics is 15–33% in those over 65 years of age and increases with age and is 50% more common in women than in men [3]. The incidence of dry eye is correlated with the level of glycated hemoglobin: the higher the level of glycated hemoglobin, the higher the incidence of dry eye [4].

The Beaver Dam Eye Study reported that approximately 20% of dry eyes occurred in individuals with Type 2 diabetes aged between 43 and 86 years. Hom and De Land reported that 53% of patients with either diabetes or borderline diabetes had self-reported, clinically relevant dry eyes [5]. In a hospital-based study, 54% of those with diabetes had DES and there was a significant correlation between DES and the duration of diabetes. This suggests that examination for dry eye should be an integral part of the ocular examination in patients with diabetes [2].

Significant associations have been identified between diabetic retinopathy (DR) and DES. In a hospital-based study, 17.1% of DES in patients with DM was found to have mild nonproliferative diabetic retinopathy (NPDR), 17.1% had moderate NPDR, 11.1% had severe nonproliferative diabetic retinopathy (NPDR), and 25.1% had proliferative diabetic retinopathy (PDR) [6]. DR is also associated with a decrease in tear film function. Tear break-up time (BUT) and Schirmer's test values were significantly decreased in the PDR group compared to the non-DR group while corneal

fluorescein staining scores, positive rate of rose Bengal staining, the surface regularity index, and the surface asymmetry index were increased. The concentrations of lactoferrin and tear-specific prealbumin were decreased in the DR group [6]. Another hospital-based study showed that DES is more prevalent in individuals with DR and/or clinically significant macular edema ($P = 0.006$) compared to the non-DR group. The odds of DR in DES were 2.29 (CI = 1.16–4.52, $P = 0.016$) and both DES and retinopathy were associated with HbA1c [7].

3. Classification of Dry Eye Syndrome

DES was recognized as a lacrimal function unit (LFU) dysfunction disease by the International Dry Eye Workshop in 2007. The LFU which protects and maintains the tear film and normal function of the ocular surface is composed of "the cornea, conjunctiva, lacrimal gland, meibomian gland, lids, and the sensory and motor nerves that connect them" [8]. Human tear film comprises three layers: lipid (secreted by the meibomian gland), aqueous (secreted by the lacrimal gland), and mucin (secreted by conjunctiva, cornea, lacrimal gland, and other structures). These three layers contain enzymes, signaling molecules, and metabolites and are essential in maintaining the physiological function of the ocular surface [9].

The 1995 NEI/Industry Dry Eye Workshop identified two types of DES: aqueous tear-deficient (tear-deficient, lacrimal tear deficiency) and evaporative dry eye. Aqueous-deficient dry eye has two major subgroups: Sjögren and non-Sjögren syndrome. Evaporative dry eye may be intrinsic (e.g., due to meibomian gland dysfunction, eyelid problems, or low blink rate) or extrinsic (e.g., due to vitamin A deficiency, preservatives in topical medications, contact lens wear, or diseases of the ocular surface) [10]. DM associated dry eye may be tear-deficient or evaporative dry eye [7].

4. Etiology of Diabetes Mellitus Associated Dry Eye Syndrome

LFU plays a regulatory role in tear secretion and tear film formation and maintains the normal physiology of the ocular surface; damage to any component of LFU leads to tear-deficient or evaporative DES.

Tear hyperosmolarity and tear film instability caused by LFU and ocular surface dysfunction are the key factors in DES. Effects of hyperglycemia on any component of the LFU may be transferred to the entire system via neural connections, leading to insufficient tear production or excess tear loss, abnormalities in blinking, and changes in tear film composition [10]; all these cause DES. The feedback loop for tear secretion and impact of diabetes mellitus on ocular surface and tear production are summarized in Figure 1.

4.1. Lacrimal Functional Unit Dysfunction. Patients with Type 1 or Type 2 Diabetes are at increased risk of developing LFU dysfunction [11].

DM is a risk factor for corneal epithelial abnormalities. DM causes epithelial barrier dysfunction which subsequently leads to corneal complications and then LFU dysfunction [11]. Diabetes with increased serum HbA1c levels is more predisposed to impaired barrier function in the corneal epithelium [11]. In a diabetic rabbit corneal epithelium dysfunction model, increased levels of glucose, glycogen, and sorbitol have been identified in the diabetic corneal epithelium as compared to controls suggesting that sorbitol pathway activation is involved [12].

The corneal complications caused by hyperglycemia include superficial punctate keratopathy, trophic ulcers, persistent epithelial defects, and recurrent corneal erosions; all these associated with DES [13]. It has also been shown that diabetics have lower values of tear secretion and tear break-up time test (TBUT).

In aC57BL/6Jdb/db mice model of DMDES, tear production substantially decreased concomitantly with a wounded corneal epithelium. Oxidative stress in the cornea was significantly increased with decreased SIRT1 expression [14]. The mean conjunctival staining scores were significantly increased in a diabetic group ($P = 0.034$) compared with a nondiabetic group [15]. The development of DES in association with antigen-specific insulitis and diabetes in a diabetes mice model has also been demonstrated [16].

4.2. Abnormal Tear Dynamics

4.2.1. Abnormal Enzyme Metabolism. Aldose reductase is an important enzyme in the pathway involved in the pathogenesis of dry eye and oral administration of aldose reductase inhibitors has been demonstrated to improve tear dynamics [17, 18]. The polyol pathway is triggered by high glucose in Type 2 diabetes, inducing the activation of aldose reductase. It has been shown that the accumulation of sorbitol within cells leads to cellular edema and dysfunction, which ultimately results in lacrimal gland structure damage and dysfunction and the induction of decreased tear secretion.

4.2.2. Decreased Mucin Secretion. In humans, mucosal and ocular surfaces are covered and protected by a high-molecular weight, heavily glycosylated protein, which is secreted by goblet cells and exogenous glands. About 20 basic types of mucins have been identified throughout the human body; at least 7 or 8 types of mucins are found in ocular surface. Tear mucin is secreted by the conjunctival goblet cells and conjunctival and corneal epithelial cells and contributes to the mucus layer. In addition to its protective effect, mucin also forms the glycocalyx that contributes to cell adhesion and makes the tear film hydrophilic. Diabetes causes corneal and conjunctival epithelial damage, inducing reduction of the number of goblet cells; it reduces mucin production and the hydrophilic nature of the ocular surface leading to tear film instability [2, 18].

4.3. Diabetic Neuropathy. Diabetic neuropathy may be an important risk factor for lacrimal gland dysfunction. Nakata

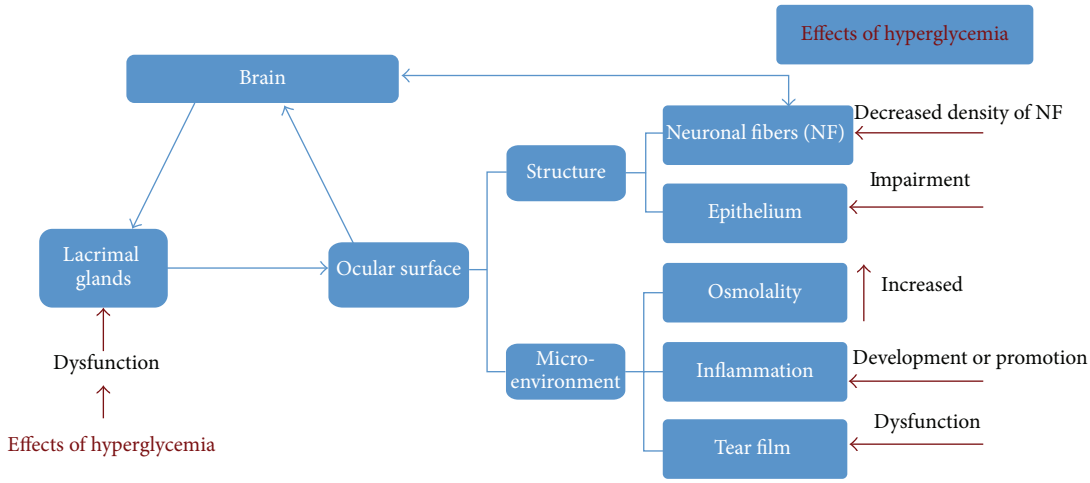

Figure 1: Lacrimal function unit (LFU) is composed of the "cornea, conjunctiva, lacrimal gland, meibomian gland, lids, and the sensory and motor nerves that connect them," which protect and maintain the tear film and normal function of the ocular surface. LFU plays a regulatory role in tear secretion and tear film formation to maintain the normal physiology of the ocular surface; damage to any component of LFU leads to tear-deficient or evaporative diabetes mellitus associated dry eye syndrome.

et al. demonstrated that diabetes suppresses hemodialysis-induced increases in tear fluid secretion, which suggests that autonomic control of lacrimal gland function may be compromised by neuropathy in patients with DM [19].

Nerve fibers play an important role in the maintenance of normal function of the cornea and the integrity of the LFU. Hyperglycemia causes corneal epithelium barrier dysfunction and corneal neuropathy, subsequently triggering the trophic effects of the cornea dysfunction [20]. Chronic sensorimotor distal symmetric polyneuropathy (PN) is the most common form of diabetic neuropathy and is characterized by sensory and motor deficits. DES is particularly common in patients with Type 2 diabetes complicated with polyneuropathy (PN) [21]. Impaired corneal neurons and reduced corneal sensitivity have been reported in diabetic patients with PN [21]. Myelinated A-δ and unmyelinated C fibers are the main neural components of the human cornea. There is a significant difference in DES between those with diabetic PN, those without diabetic PN, and control subjects. The values of Schirmer's I test, TBUT, and corneal sensitivity were also worse in patients with PN compared to diabetics without PN and normal controls ($P < 0.001$) [18]. These findings suggest that patients with PN should be considered for testing for DES to prevent ocular surface impairment during the follow-up.

4.4. Tear Film Dysfunction. The tear film is the most dynamic structure of the LFU. It plays an important role in regulating epithelium function and interacting with surrounding tissues [22]. Tear film dysfunction has been found to be closely associated with DES. Chronic tear secretion deficiency and tear film dysfunction have also been identified in patients with diabetes [23, 24]. The tear lipid thickness (especially the lipid layer of the tear film), stability, corneal sensitivity, and tear quantity were significantly decreased in patients with diabetes. Tear film stability was inversely associated with the total neuropathy score [25].

5. Pathogenesis of DM Associated Dry Eye Syndrome

Chronic hyperglycemia, diabetic periphery neuropathy, decreased insulin levels, microvasculopathy, and systemic hyperosmotic disturbances are risk factors for diabetes-associated DES (Figure 2).

Insulin is critical for proliferation of the acinar lacrimal gland (LG) and cornea epithelial cells. Insulin partially reversed the decreased protein expression induced by LG dysfunction; this process is involved in supporting exocytosis and vesicular formation through insulin replacement therapy [26]. It has been demonstrated that hyperglycemia induces histological alterations in the lacrimal gland, suggesting the role of diabetes-induced oxidative stress in DES [27]. Significant decreased reflex tearing was also reported in insulin dependent diabetic patients [23].

The glucose level is increased in the tears of diabetic patients [14]. A high glucose level in diabetic patients leads to elevated expression level of advanced glycation end-product-(AGE-) modified proteins. AGE-modified proteins in tears may be used as biomarkers to diagnose diabetes and/or DR [28].

Inflammation and immunity have been shown to play a prominent role in the pathogenesis of DES. Hyperglycemia initiates an inflammatory cascade that generates innate and adaptive immune responses of LFU. The downstream immune-inflammatory regulators have been identified to include matrix metalloproteinase-9 (MMP-9), immature antigen-presenting cells (APCs), $CD4^+$ helper T cell (T_H) subtype 1 and T_H17 cell subsets, interferon (IFN) γ chemokines, chemokine receptors, cell adhesion

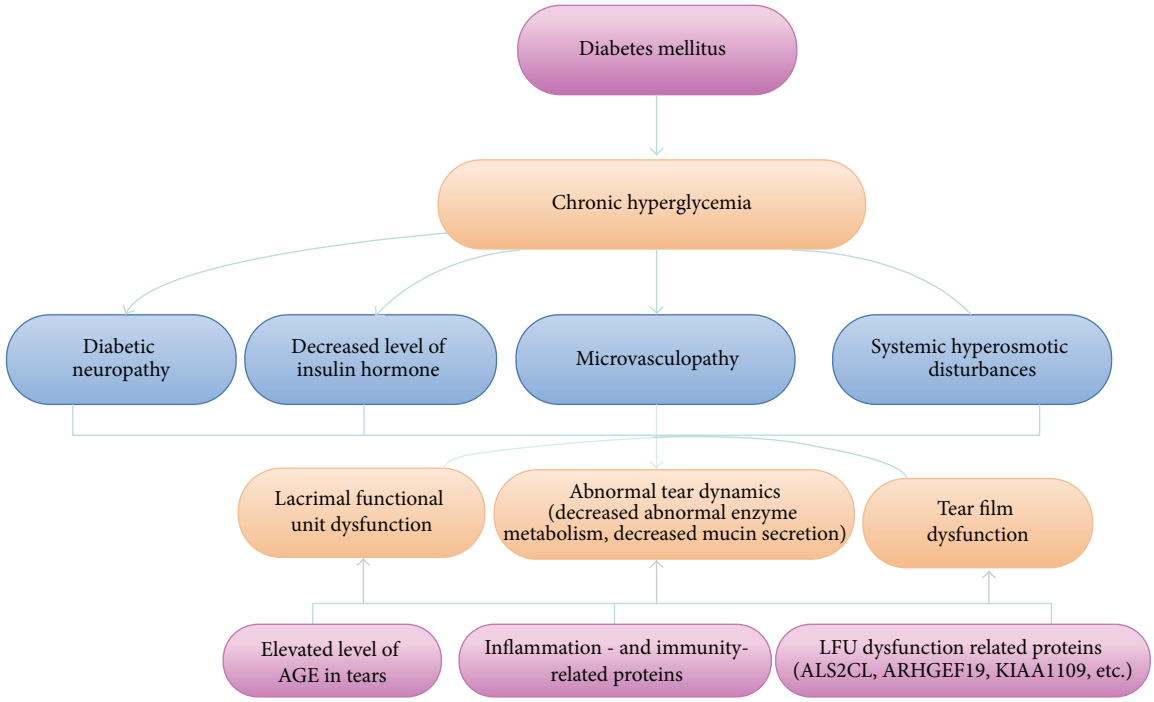

Figure 2: Etiology and pathogenesis of diabetes mellitus associated dry eye syndrome. Chronic hyperglycemia, diabetic periphery neuropathy, decreased insulin hormone, microvasculopathy, and systemic hyperosmotic disturbances are risk factors for diabetes-associated dry eye syndrome, which subsequently induce lacrimal function unit and tear film dysfunction and abnormal tear dynamics. Several proteins have been identified to be the contributor to diabetes mellitus associated dry eye syndrome.

molecules (CAMs), and interleukin-17 (IL-17) [29]. Furthermore, hyperglycemia causes tear film hyperosmolarity, inducing hyperosmolarity of the ocular surface epithelial cells, and stimulates a cascade of inflammatory events that involve MAP kinases and NFkB signaling pathways. The generation of inflammatory cytokines (e.g., interleukin-1A (IL-1A) and interleukin-1B (IL-1B), tumor necrosis factor-A (TNF-A), and matrix metalloproteinase-9 (MMP-9)) has also been demonstrated to be involved in the pathogenesis of DES [10, 30, 31].

Thousands of proteins have been identified and may be responsible for LFU dysfunction. Proteins (expressed in the human LFU) which are involved in the pathogenesis of diabetic DES include ALS2CL, ARHGEF19, KIAA1109, PLXNA1, POLG, WIPI1, ZMIZ2, and lacritin. The role of these proteins in patients with diabetic DES warrants further study [7]. The expression of apoptosis-related proteins, such as annexin A1, immunity- and inflammation-related proteins, including neutrophil elastase 2 and clusterin, and glycometabolism-related proteins, such as apolipoprotein A-II, has been reported to be increased in patients with DMDES [32].

6. Clinical Characteristics of Diabetes Mellitus Associated Dry Eye Syndrome

Diabetic patients with dry eye may have the same symptoms as DES without diabetes [2]. The symptoms consist of a gritty sensation, soreness, decreased visual acuity, photophobia, itching, decreased goblet cell density and corneal sensitivity, and tearing and pain concomitant with abnormalities in TUBUT, Schirmer's test, and corneal staining. More severe cases may be complicated by corneal lesions, conjunctivitis, keratopathy, and inflammation. It has been reported that gritty sensation is the most prominent symptom followed by the abnormalities of the tear film in patients with DMDES [2].

Dry eye symptoms are typically severe in patients with diabetes whose glycemic control is poor [33, 34]. Those with longer duration of diabetes may report fewer dry eye symptoms [16], and increased tear osmolarity is negatively correlated with symptoms. However, those without symptoms are unlikely to seek care. Lack of symptoms may result from a reduction in corneal sensitivity caused by diabetic peripheral corneal neuropathy [35]. Even a minimal decrease in corneal sensitivity is sufficient to cause changes in tear secretion. In a hospital-based study, longer duration of diabetes was associated with a lower (less severe) ocular surface disease index [2].

BUT (or NIBUT) and the Schirmer test are the most applied clinical methods used to diagnose DES. Tear osmolality and dynamics may also be used as supplementary diagnostic methods. In patients with diabetes, routine examination with BUT and Schirmer test is recommended. Early intervention is important to avoid visual impairment.

7. Prevention and Treatment Regimens of Diabetes Mellitus Associated Dry Eye Syndrome

Severe DMDES leads to visual impairment, corneal scarring, and ulcers, leading to secondary bacterial infections. The synergistic effect of corneal infection and diabetes accelerates corneal lesions, which irreversibly change the ocular surface and induce visual impairment [36]. Tear film dysfunction not only leads to the occurrence of dry eye but simultaneously aggravates the ocular surface, which induces a corneal epithelial defect, a common sign in diabetics [37].

The early diagnosis and treatment of dry eye are essential to avoid complications. The current treatment regimens for diabetic and nondiabetic dry eye patients are essentially the same. To date, there is no unified treatment option for DES. The application of artificial tears, including surfactants and various viscous agents, is predominately used to improve symptoms [38]. Artificial tears temporarily improve blurred vision and other symptoms. The drugs with anti-inflammatory effects do not comprise the active components such as growth factors which are contained in normal human tears [39, 40].

The most widely used anti-inflammatory drugs are corticosteroids, nonsteroidal anti-inflammatory drugs, cyclosporin A, tacrolimus, autologous blood serum, and several new drugs which are undergoing clinical trials [39, 41]. In patients with DMDES, corneal epithelial defects or side effects correlated with the topical drugs are more common than in those DES patients without DM; frequent routine follow-up for DMDES is necessary during treatment. Some devices are under development to help release the symptoms [42].

Topical corticosteroids reduce the signs, symptoms, and the level of inflammation in dry eyes and prevent corneal epithelial damage [43]. The ocular surface disease index score and dendritic cell density significantly improved by topical corticosteroids treatment [44]. The mechanisms of actions of corticosteroids on DES may be through suppression of cellular infiltration and increased synthesis of lipocortin which in turn block phosphorylation of phospholipase A2, which is the key step of the inflammatory cascade [41, 45]. However, side effects such as bacterial and fungal infections, increase in intraocular pressure, and cataracts have been reported [46]. Application of lower concentration of the steroids in short duration (one or two weeks) of the tropical steroid drug is recommended for those patients with DMDES.

To avoid side effects of topical steroids, nonsteroidal anti-inflammatory drugs (NSAIDs) are more commonly used instead of steroids in the clinic [47]. Pranoprofen, Bromfenac Sodium Hydrate, and RESTASIS® containing 0.05% cyclosporine have been applied in clinical practice. These topic drugs increase tear production, suppress immune response, and reduce damage to goblet cells induced by inflammation [48]. These drugs relieve the symptoms of aqueous-deficient dry eye and promote corneal epithelial recovery, but they do not improve tear production. Furthermore, these drugs reduce the sensitivity of the cornea, leading to corneal epithelium dissolution; they are recommended to be carefully applied to DM patients.

The mechanism of action of tacrolimus is similar to cyclosporin A, but the anti-inflammatory effect is stronger than that of cyclosporin A. It suppresses inflammation by inhibition of the expression of inflammation cytokines and chemokines [41, 49, 50].

Autologous blood serum eye drops have been shown to be effective on DES [51]. They contain immunoglobulins, vitamin A, fibronectin, growth factors, and anti-inflammatory cytokines which are the essential components present in natural tears. It has been found that 50% of the autologous serum eye drops are safe and effective for severe dry eye which is resistant to all other conventional treatments in a retrospective cohort study [52]. It has also been demonstrated that autologous serum tears are beneficial in the treatment of persistent corneal epithelial defect [53]. However, autologous serum tears do not have preservatives; they have a potential risk of inducing secondary infections; therefore, attention needs to be paid during the treatment, especially for those patients with DMDES.

Several drugs such as chemokine receptor antagonist, tofacitinib, LFA-1 antagonist, rebamipide (quinolinone derivative mucin secretagogue), MiM-D3 (nerve growth factor peptidomimetic, mucin secretagogue), EBI 005 (eleven biotherapeutics), diquafosol (P2Y2 receptor agonist), RU-101 (recombinant human serum albumin), KPI-121/LE-MMP 0.25%, and lifitegrast 5% (a small-molecule integrin antagonist) are undergoing clinical trials [41, 42]. Gene therapies that target LG have been demonstrated to be an alternative method in animal models of dry eye and specific treatment based on the pathogenesis of the condition in diabetic patients with dry eye warrants additional research [54].

In clinical practice, diabetics undergo regular fundus examinations. It has been suggested that the examination of the ocular surface and tear function also become part of the routine diabetic ophthalmic assessment and follow-up. Furthermore, preservative-free artificial tears and anti-inflammatory drugs are recommended to improve the hyperosmolar state of tears and to reduce the local inflammatory reaction. Protection of cornea and prevention of DMDES need to be considered in patients with islet dysfunction or poor glycemic control.

In summary, increasing prevalence of DMDES has been reported in recent years. In addition to the DR concerned, which is the leading cause of blindness, more attention should be paid to DMDES, the most frequent diabetic complication in eye disorders in clinical practice. The pathogenesis of diabetes-related DES remains elusive, and limited specific interventions are currently available. Additional clinical trials are warranted to confirm the effects of the currently applied drugs in diabetes-associated DES. Moreover, with the development of biomedical research, additional drugs, as well as gene and stem cell therapies, with specific targets will become available for the treatment of DES in diabetes.

Abbreviations

AGE: Advanced glycation end-product
APCs: Antigen-presenting cells
BUT: Break-up time
CAMs: Cell adhesion molecules
DES: Dry eye syndrome
DM: Diabetes mellitus
DMDES: Diabetes mellitus associated dry eye syndrome
DR: Diabetic retinopathy
IFN: Interferon
IL: Interleukin
LFU: Lacrimal function unit
LG: Lacrimal gland
MMP-9: Matrix metalloproteinase-9
NPDR: Nonproliferative diabetic retinopathy
PDR: Proliferative diabetic retinopathy
PN: Polyneuropathy
TBUT: Tear break-up time test
TNF: Tumor necrosis factor.

Competing Interests

The authors declare that they have no competing interests.

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References

[1] Y. Xu, "Prevalence and control of diabetes in Chinese adults," *The Journal of the American Medical Association*, vol. 310, no. 9, pp. 948–959, 2013.

[2] M. R. Manaviat, M. Rashidi, M. Afkhami-Ardekani, and M. R. Shoja, "Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients," *BMC Ophthalmology*, vol. 8, article 10, 2008.

[3] Dry eye syndrome, NICE CKS, September 2012 (UK access only).

[4] U. Seifart and I. Strempel, "The dry eye and diabetes mellitus," *Ophthalmologe*, vol. 91, no. 2, pp. 235–239, 1994.

[5] M. Hom and P. De Land, "Self-reported dry eyes and diabetic history," *Optometry*, vol. 77, no. 11, pp. 554–558, 2006.

[6] J. Nepp, C. Abela, I. Polzer, A. Derbolav, and A. Wedrich, "Is there a correlation between the severity of diabetic retinopathy and keratoconjunctivitis sicca?" *Cornea*, vol. 19, no. 4, pp. 487–491, 2000.

[7] R. L. McKown, N. Wang, R. W. Raab et al., "Lacritin and other new proteins of the lacrimal functional unit," *Experimental Eye Research*, vol. 88, no. 5, pp. 848–858, 2009.

[8] G. Rieger, "The importance of the precorneal tear film for the quality of optical imaging," *British Journal of Ophthalmology*, vol. 76, no. 3, pp. 157–158, 1992.

[9] M. A. Lemp, "Advances in understanding and managing dry eye disease," *American Journal of Ophthalmology*, vol. 146, no. 3, pp. 350.e1–356.e1, 2008.

[10] "Research in dry eye: report of the ResearchSubcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 179–193, 2007.

[11] M. Gekka, K. Miyata, Y. Nagai et al., "Corneal epithelial barrier function in diabetic patients," *Cornea*, vol. 23, no. 1, pp. 35–37, 2004.

[12] J. Friend, T. C. Kiorpes, and R. A. Thoft, "Diabetes mellitus and the rabbit corneal epithelium," *Investigative Ophthalmology and Visual Science*, vol. 21, no. 2, pp. 317–321, 1981.

[13] "Vaughan and Asbury's general ophthalmology, 17th edition," *Clinical and Experimental Optometry*, vol. 91, no. 6, pp. 577–577, 2008.

[14] H. Liu, M. Sheng, Y. Liu et al., "Expression of SIRT1 and oxidative stress in diabetic dry eye," *International Journal of Clinical and Experimental Pathology*, vol. 8, no. 6, pp. 7644–7653, 2015.

[15] K. A. Beckman, "Characterization of dry eye disease in diabetic patients versus nondiabetic patients," *Cornea*, vol. 33, no. 8, pp. 851–854, 2014.

[16] S. Imam, R. B. Elagin, and J. C. Jaume, "Diabetes-associated dry eye syndrome in a new humanized transgenic model of type 1 diabetes," *Molecular Vision*, vol. 19, pp. 1259–1267, 2013.

[17] C. Ramos-Remus, M. Suarez-Almazor, and A. S. Russell, "Low tear production in patients with diabetes mellitus is not due to Sjogren's syndrome," *Clinical and Experimental Rheumatology*, vol. 12, no. 4, pp. 375–380, 1994.

[18] S. C. G. Tseng, L. W. Hirst, and A. E. Maumenee, "Possible mechanisms for the loss of goblet cells in mucin-deficient disorders," *Ophthalmology*, vol. 91, no. 6, pp. 545–552, 1984.

[19] M. Nakata, Y. Okada, H. Kobata et al., "Diabetes mellitus suppresseshemodialysis-induced increases in tear fluid secretion," *BMC Research Notes*, vol. 7, no. 1, article 78, 2014.

[20] R. A. Hyndiuk, E. L. Kazarian, R. O. Schultz, and S. Seideman, "Neurotrophic corneal ulcers in diabetes mellitus," *Archives of Ophthalmology*, vol. 95, no. 12, pp. 2193–2196, 1977.

[21] V. Achtsidis, I. Eleftheriadou, E. Kozanidou et al., "Dry eye syndrome in subjects with diabetes and association with neuropathy," *Diabetes Care*, vol. 37, no. 10, pp. e210–e211, 2014.

[22] M. Rolando and M. Zierhut, "The ocular surface and tear film and their dysfunction in dry eye disease," *Survey of Ophthalmology*, vol. 45, supplement 2, pp. S203–S210, 2001.

[23] M. Goebbels, "Tear secretion and tear film function in insulin dependent diabetics," *British Journal of Ophthalmology*, vol. 84, no. 1, pp. 19–21, 2000.

[24] M. Dogru, C. Katakami, and M. Inoue, "Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus," *Ophthalmology*, vol. 108, no. 3, pp. 586–592, 2001.

[25] S. L. Misra, D. V. Patel, C. N. J. McGhee et al., "Peripheral neuropathy and tear film dysfunction in type 1 diabetes mellitus," *Journal of Diabetes Research*, vol. 2014, Article ID 848659, 6 pages, 2014.

[26] A. C. Dias, T. M. Batista, L. P. Roma et al., "Insulin replacement restores the vesicular secretory apparatus in the diabetic rat lacrimal gland," *Arquivos Brasileiros de Oftalmologia*, vol. 78, no. 3, pp. 158–163, 2015.

[27] C. M. Módulo, A. G. Jorge, A. C. Dias et al., "Influence of insulin treatment on the lacrimal gland and ocular surface of diabetic rats," *Endocrine*, vol. 36, no. 1, pp. 161–168, 2009.

[28] Z. Zhao, J. Liu, B. Shi, S. He, X. Yao, and M. D. P. Willcox, "Advanced glycation end product (AGE) modified proteins in tears of diabetic patients," *Molecular Vision*, vol. 16, pp. 1576–1584, 2010.

[29] W. Stevenson, "Dry eye disease," *Archives of Ophthalmology*, vol. 130, no. 1, pp. 90–100, 2012.

[30] L. Luo, D.-Q. Li, R. M. Corrales, and S. C. Pflugfelder, "Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface," *Eye and Contact Lens*, vol. 31, no. 5, pp. 186–193, 2005.

[31] D.-Q. Li, L. Luo, Z. Chen, H.-S. Kim, X. J. Song, and S. C. Pflugfelder, "JNK and ERK MAP kinases mediate induction of IL-1β, TNF-α and IL-8 following hyperosmolar stress in human limbal epithelial cells," *Experimental Eye Research*, vol. 82, no. 4, pp. 588–596, 2006.

[32] B. Li, M. Sheng, L. Xie et al., "Tear proteomic analysis of patients with type 2 diabetes and dry eye syndrome by two-dimensional nano-liquid chromatography coupled with tandem mass spectrometry," *Investigative Ophthalmology and Visual Science*, vol. 55, no. 1, pp. 177–186, 2014.

[33] M. Ozdemir, M. A. Buyukbese, A. Cetinkaya, and G. Ozdemir, "Risk factors for ocular surface disorders in patients with diabetes mellitus," *Diabetes Research and Clinical Practice*, vol. 59, no. 3, pp. 195–199, 2003.

[34] I. Kaiserman, N. Kaiserman, S. Nakar, and S. Vinker, "Dry eye in diabetic patients," *American Journal of Ophthalmology*, vol. 139, no. 3, pp. 498–503, 2005.

[35] N. Fuerst, N. Langelier, M. Massaro-Giordano et al., "Tear osmolarity and dry eye symptoms in diabetics," *Clinical Ophthalmology*, vol. 8, pp. 507–515, 2014.

[36] A. J. Lubniewski, K. W. Houchin, E. J. Holland et al., "Posterior infectious crystalline keratopathy with *Staphylococcus epidermidis*," *Ophthalmology*, vol. 97, no. 11, pp. 1454–1459, 1990.

[37] K. Inoue, S. Kato, C. Ohara, J. Numaga, S. Amano, and T. Oshika, "Ocular and systemic factors relevant to diabetic keratoepitheliopathy," *Cornea*, vol. 20, no. 8, pp. 798–801, 2001.

[38] R. M. Schiffman, M. D. Christianson, G. Jacobsen, J. D. Hirsch, and B. L. Reis, "Reliability and validity of the ocular surface disease index," *Archives of Ophthalmology*, vol. 118, no. 5, pp. 615–621, 2000.

[39] "Management and therapy of dry eye disease: report of the Management and TherapySubcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 163–178, 2007.

[40] W. B. Jackson, "Management of dysfunctional tear syndrome: a Canadian consensus," *Canadian Journal of Ophthalmology*, vol. 44, no. 4, pp. 385–394, 2009.

[41] M. Hessen and E. K. Akpek, "Dry eye: an inflammatory ocular disease," *Journal of Ophthalmic and Vision Research*, vol. 9, no. 2, pp. 240–250, 2014.

[42] L. A. Vickers and P. K. Gupta, "The future of dry eye treatment: a glance into the therapeutic pipeline," *Ophthalmology and Therapy*, vol. 4, no. 2, pp. 69–78, 2015.

[43] C.-Q. Yang, W. Sun, and Y.-S. Gu, "A clinical study of the efficacy of topical corticosteroids on dry eye," *Journal of Zhejiang University SCIENCE B*, vol. 7, no. 8, pp. 675–678, 2006.

[44] E. Villani, E. Garoli, V. Termine, F. Pichi, R. Ratiglia, and P. Nucci, "Corneal confocal microscopy in dry eye treated with corticosteroids," *Optometry and Vision Science*, vol. 92, no. 9, pp. e290–e295, 2015.

[45] T. L. Comstock and H. H. Decory, "Advances in corticosteroid therapy for ocular inflammation: loteprednol etabonate," *International Journal of Inflammation*, vol. 2012, Article ID 789623, 11 pages, 2012.

[46] N. Sayin, N. Kara, and G. Pekel, "Ocular complications of diabetes mellitus," *World Journal of Diabetes*, vol. 6, no. 1, pp. 92–108, 2015.

[47] B. Colligris, H. A. Alkozi, and J. Pintor, "Recent developments on dry eye disease treatment compounds," *Saudi Journal of Ophthalmology*, vol. 28, no. 1, pp. 19–30, 2014.

[48] X. Q. Zhou and R. L. Wei, "Topical cyclosporine A in the treatment of dry eye: a systematic review and meta-analysis," *Cornea*, vol. 33, no. 7, pp. 760–767, 2014.

[49] S. Reitamo, A. Remitz, H. Kyllönen, and J. Saarikko, "Topical noncorticosteroid immunomodulation in the treatment of atopic dermatitis," *American Journal of Clinical Dermatology*, vol. 3, no. 6, pp. 381–388, 2002.

[50] S. Aoki, "Systemic FK506 improved tear secretion in dry eye associated with chronic graft versus host disease," *British Journal of Ophthalmology*, vol. 89, no. 2, pp. 243–244, 2005.

[51] I. Katsakoulas, C. Lougovoi, P. Paraskevopoulou, and N. Vougioukas, "Protocol of blood serum eye drops," *International Journal of Pharmaceutical Compounding*, vol. 19, no. 3, pp. 252–260, 2014.

[52] M. Hussain, R. M. Shtein, A. Sugar et al., "Long-term use of autologous serum 50% eye drops for the treatment of dry eye disease," *Cornea*, vol. 33, no. 12, pp. 1245–1251, 2014.

[53] A. L. Young, A. C. O. Cheng, H. K. Ng, L. L. Cheng, G. Y. S. Leung, and D. S. C. Lam, "The use of autologous serum tears in persistent corneal epithelial defects," *Eye*, vol. 18, no. 6, pp. 609–614, 2004.

[54] E. M. Rocha, G. Di Pasquale, P. P. Riveros, K. Quinn, B. Handelman, and J. A. Chiorini, "Transduction, tropism, and biodistribution of AAV vectors in the lacrimal gland," *Investigative Ophthalmology and Visual Science*, vol. 52, no. 13, pp. 9567–9572, 2011.

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Research Article

Hyaluronate Acid-Dependent Protection and Enhanced Corneal Wound Healing against Oxidative Damage in Corneal Epithelial Cells

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Purpose. To evaluate the effects and mechanism of exogenous hyaluronate (HA) in promoting corneal wound healing. *Methods*. Human corneal epithelial cells (HCECs) were incubated with different concentrations of HA to evaluate their efficiency in promoting cell migration and their modulation of repair factors. After inducing hyperosmolar conditions, the cell morphologies, cell apoptosis, and expression levels of TNF-α and MMP-9 were detected to assess the protective role of HA. Corneal epithelium-injured rat models were established to test the therapeutic effects of 0.3% HA. Then, the wound healing rates, the RNA expression levels of inflammatory cytokines, and repair factors were examined. *Results*. HCECs in the 0.03% and 0.3% HA groups showed fewer morphological alterations and lower rates of cell apoptosis following preincubation with HA under hyperosmolar conditions, as well as the expression levels of MMP-9 and TNF-α. In the rat model, the areas of fluorescein staining in the corneas of 0.3% HA group were significantly smaller than the control group. The expression levels of IL-1β and MMP-9 were decreased, while CD44 and FN were increased in the 0.3% HA group. *Conclusion*. HA enhanced corneal epithelial cell wound healing by promoting cell migration, upregulating repair responses, and suppressing inflammatory responses.

1. Introduction

A healthy corneal epithelium is essential to protect the eye against infection and structural damage [1]. The conditions/factors that most commonly lead to epithelial defects include epithelial stem cell deficiency, inflammatory diseases, neurotrophic diseases, and mechanical factors [2–4]. Several clinical treatments are used for epithelial defects, including lubrication, punctual plugs, bandage contact lenses, and tarsorrhaphy [5, 6]. Specifically, artificial tears represent one of the most important interventions for lubricating the ocular surface, supplementing insufficient tears, diluting inflammatory cytokines, and reducing the tear osmotic pressure, which has the potential to induce cell apoptosis [7, 8].

Hyaluronate is an excellent representative of artificial tears, and it is used to alleviate ocular discomfort, prolong tear stability, and promote corneal epithelial repair [9]. It is a glycosaminoglycan found in various connective tissues, such as epithelial and neural tissues, and it interacts with water to dilate the extracellular matrix and acts as a lubricant to assist in cell migration [10]. According to the published reports, its concentration increases during the process of wound repair, and it is used as an exogenous intervention to promote this process [11]. The role of HA as a key component of the extracellular matrix structure has been recognized for many decades [12], while its actions on cells involved in corneal epithelial repair have been determined in part only in the last few years [13].

Hyaluronate, as well as its degradation products that are generated during corneal epithelial repair, is capable of activating specific intracellular responses, of which epithelial proliferation, cell apoptosis, inflammatory responses, and neovascularization have been exclusively examined by

in vitro studies [14, 15]. The molecular mechanisms leading to cell activation have been substantially clarified, and it is now widely accepted that the cellular actions of hyaluronate are mediated by specific surface receptors, including CD44, Fibronectin, RHAMM, and Toll-like receptors [16, 17]. In 1996, Miyazaki et al. reported that hyaluronate binds to the CD44-like molecule associated with FN and enhances the growth of corneal epithelial cells [12]. The latest reports published in 2015 have demonstrated that hyaluronate stimulates the reepithelialization of corneal wounds *in vitro* [4]. However, the mechanism and effects of exogenous hyaluronate remain to be further elucidated, which will allow for the optimization of its clinical use.

Our study aimed to examine the characteristics of hyaluronate and the process of corneal epithelial cell repair to elucidate the mechanism by which exogenous hyaluronate promotes the healing of injured corneal epithelial cells and to determine the optimal concentration of hyaluronate for clinical use. Moreover, we generated a corneal epithelium-injured animal model to analyse the therapeutic effects and mechanism of hyaluronate and to provide experimental and theoretic evidence for managing corneal epithelial defects clinically.

2. Materials and Methods

2.1. Cell Culture. The human corneal epithelial cell (HCEC) line was a gift from Professor ZhiChong Wang (Zhongshan Ophthalmic Centre, Guangzhou, China). The cell line was maintained in Dulbecco's-modified Eagle's medium (Gibco BRL) supplemented with 15% foetal bovine serum (Gibco BRL), 10 ng/mL human EGF (Gibco BRL), 5 mg/mL insulin, 5 mg/mL human transferase (Sigma), 0.4 mg/mL hydrocortisone (Gibco BRL), 0.1 mm 2-mercaptoethanol (Gibco BRL), 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin (HyClone; corneal epithelial cell culture medium).

2.2. Scratch Wound Healing Assay. HCECs were trypsinized and seeded at a density of 10,000 cells per well into 24-well plates that contained a culture insert. Then, scratch wounds and premarked lines were created after a 24 h incubation period. After the removal of the debris resulting from the linear scratching, HCEC monolayers were divided into the following three groups: a control group, which was incubated with serum-free medium for 48 h; a 0.03 HA group, which was incubated with serum-free medium containing hyaluronate at a final concentration of 0.03% for 48 h; and a 0.3 HA group, which was incubated with serum-free medium containing hyaluronate at a final concentration of 0.3% for 48 h. Cell proliferation was recorded using an inverted microscope, and images were captured to visualize the interactions between the scratched wound areas and premarked lines. Then, the protein levels of the repair factors FN and CD44 were measured by ELISA.

2.3. Cell Challenge Conditions. HCECs were trypsinized and seeded at a density of 10,000 cells per well into 24-well plates. Then, they were divided into three groups for exposure to different concentrations of HA as follows: control (no HA), 0.03 HA, and 0.3 HA groups (as mentioned above). Next, a combination of hyperosmolar solution (500 mOsM) and BAK (90 mM) was added to the medium to generate hyperosmolar conditions. Cells were monitored using a microscope with a camera attached to detect morphological changes, including decreased cell size, membrane blebbing, the formation of apoptotic bodies, and cell detachment, at 0 min, 5 min, and 15 min. Then, flow cytometry was conducted to examine the apoptosis rate, and real-time PCR was performed to measure the RNA expression levels of inflammatory cytokines, such as MMP-9 and TNF-α.

2.4. Animals. A total of 70 adult male Sprague-Dawley rats weighing approximately 250 g were purchased from the Animal Supply Centre of Sun Yat-sen University, Zhongshan School of Medicine. All procedures followed in this study were in accordance with the principles of the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

2.5. Corneal Epithelium-Injured Animal Models. The rats were anesthetized via intraperitoneal (i.p.) administration of kessodrate (10% chloral hydrate, 250 mg/kg) and were then placed beneath a stereoscopic microscope at 20x magnification. The right cornea of each rat was burned by a 3 mm^2 round filter paper soaked with 100% heptanol; the filter paper was placed on the centre of the right cornea for 40 s, and then the cornea was rinsed with normal saline for 60 s. After injury, 70 rats were divided into 2 groups: a control group, in which the rats received normal saline eye drops (20 μL) 4 times/d in the right eye, and a 0.3 HA group, in which the rats received 0.3% hyaluronate eye drops (20 μL) 4 times/d in the right eye. The burned cornea of each animal was photographed on each day after injury to record disease progression and to analyse the healing rate. Real-time PCR and ELISA were performed to determine the expression levels of inflammatory cytokines (IL-1β and MMP-9) and repair factors (FN and CD44).

2.6. Real-Time PCR. Total RNA was isolated from individual corneas using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations, and it was quantitated using a NanoDrop 2000C spectrophotometer (Thermo Scientific, West Palm Beach, FL). One microgram of total RNA was reverse transcribed to produce cDNA, and the cDNA was amplified using SYBR Green Master Mix (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Primers for human MMP-9, human TNF-α, rat IL-1β, rat MMP-9, rat FN, and rat CD44 were purchased from SABiosciences (Frederick, MD), and the primer sequences are listed in Table 1. Quantitative real-time PCR was performed using a CFX96 real-time PCR system (Bio-Rad). Relative gene expression levels were calculated after normalization to the internal control β-actin.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA). Cytokine protein levels were selectively measured using ELISA kits

Table 1: Nucleotide sequences of the specific primers used for PCR amplification.

Gene	Primer sequence (5′-3′)	
hMMP-9	AGGACAAAGCAGGATCACAGTT	F
	CCTGGGCAGATTCCAAACCT	R
hTNF-α	ATC AAT CGG CCC GAC TAT CTC	F
	GCA ATG ATC CCA AAG TAG ACC	R
hβ-actin	GCT CCT CCT GAG CGC AAG	F
	CAT CTG CTG GAA GGT GGA CA	R
rIL-1β	CAT CTT TGA AGA AGA GCC CG	F
	GGG ATT TTG TCG TTG CTT GT	R
rMMP-9	CTTTGGGCTGCCCAACACACA	F
	GAAGCAGAATTTGCGGAGGTTTT	R
rFN	GAC CTG CAA GCC AAT AGC TGA GA	F
	TCG CCC AGA CAA GTA CAG TCC A	R
rCD44	GGA ATC AAG ACA GTG GAG TGA CCA CA	F
	GAC AGC AAT GCA GAC GGC AAG AAT	R
rGAPDH	AAT GCA TCC TGC ACC ACC AA	F
	TCA CGC CAC AGC TTT CCA GA	R

(R&D Systems). For the *in vitro* experiments, HCEC supernatants were collected at 15 min. For the *in vivo* experiments, corneal samples were individually collected (n = 5/group/time) from the rats in the different groups at 1, 2, and 7 d after injury and homogenized in 0.5 mL PBS containing 0.1% Tween-20. All samples were collected and centrifuged at 13,000 rpm for 5 min, and the supernatants were collected. An aliquot of each supernatant was assayed in duplicate for measurements of the human FN and human CD44 levels, according to the manufacturer's instructions. The reported sensitivities of these assays are 0.6 ng/mL for human FN and 78.1 pg/mL for human CD44.

2.8. Flow Cytometry. Cell apoptosis was assessed by flow cytometry using an annexin V-fluorescein isothiocyanate apoptosis detection kit (BD) according to the manufacturer's instructions. Briefly, cells were pooled, washed, and resuspended in 500 μL binding buffer, followed by the addition of 5 μL annexin V-fluorescein isothiocyanate and 5 μL PI. Then, the cells were incubated at room temperature away from light for 15 min and were subsequently analysed by flow cytometry (Beckman Coulter EPICS XL/MCL). Viable cells did not exhibit annexin V or PI staining, early apoptotic cells showed annexin V but not PI staining, and late apoptotic cells exhibited both annexin V and PI staining.

2.9. Statistical Analysis. Unpaired, two-tailed Student's t-test was used to determine the statistical significance of the ELISA and real-time PCR results and the corneal epithelium healing rates. The data were considered statistically significant at $p < 0.05$.

3. Results

3.1. Hyaluronate Promotes Cell Migration and Increases the Expression of Repair Factors. To determine the role of hyaluronate in cell migration, a culture insert was placed into culture dishes containing HCECs to form a gap. Then, different concentrations of hyaluronate were added to the culture medium, and the healing effects were observed using an inverted microscope. As shown in Figure 1(a), the gap was shortened gradually from 48 h after injury in the control group. In addition, the 0.03 HA group had a higher rate of cell proliferation, and an apparent new cell mass was observed at 36 h after injury, and the 0.3 HA group exhibited the highest proliferation rate, the formation of a new cell mass at 12 h after injury, and the highest rate of new cell formation at 48 h after injury.

Moreover, we determined the protein expression levels of Fibronectin (FN) and CD44. The mean FN protein expression level (Figure 1(b)) in the control group was 63.5 ng/mL, and it increased to 127 ng/mL in the 0.03 HA group and to 162 ng/mL in the 0.3 HA group, which was 2 times more than that in the control group ($p < 0.05$). The mean CD44 protein expression levels (Figure 1(c)) were 2.92, 3.77, and 3.92 ng/mL in the control, 0.03 HA, and 0.3 HA groups, respectively. The mean CD44 protein expression level was the highest in the 0.3 HA group ($p < 0.001$). The results shown in Figure 1 indicate that hyaluronate efficiently promoted wound closure and increased the expression of repair factors, especially in the 0.3 HA group.

3.2. Hyaluronate Decreases BAK-Induced Cell Apoptosis and the Expression of Inflammatory Cytokines. To explore the potential protective role of hyaluronate in BAK-induced cell apoptosis, NaCl and BAK were added to the medium to generate hyperosmolar conditions, and changes in cell morphology were observed using a microscope. As shown in Figure 2(a), cells in the control group exhibited clear decreases in cell size and cytoplasmic retraction after 5 min of hyperosmolar stimulation, those in the 0.03 HA group were inactivated and round in size at 15 min, and those in the 0.3 HA group were still relatively active at 15 min. Moreover, flow cytometry (Figure 2(b)) revealed that the 0.3 HA group had the lowest cell apoptosis rate (12.7% early apoptotic cells and 4.82% late apoptotic cells), followed by the 0.03 HA group (17.8% early apoptotic cells and 5.50% late apoptotic cells), and it revealed that the control group had the largest number of apoptotic cells (46.9% early apoptotic cells and 3.29% late apoptotic cells). Real-time PCR (Figures 2(c) and 2(d)) demonstrated that stimulation by BAK led to the upregulation of the expression of inflammatory cytokines, such as MMP-9 and TNF-α, compared with the control group. Hyaluronate downregulated their expression, and the 0.3 HA group showed the lowest inflammatory cytokine levels among the three groups. Taken together, these results revealed that hyaluronate facilitated reductions in cell apoptosis and inflammatory response induced by the hyperosmolar conditions.

3.3. Hyaluronate Accelerates Corneal Epithelial Wound Healing in a Heptanol-Burned Model. Based on the results of the *in vitro* study, we have demonstrated hyaluronate role in promoting corneal epithelial cell proliferation, and we have shown that higher concentrations of HA (0.3% HA) have

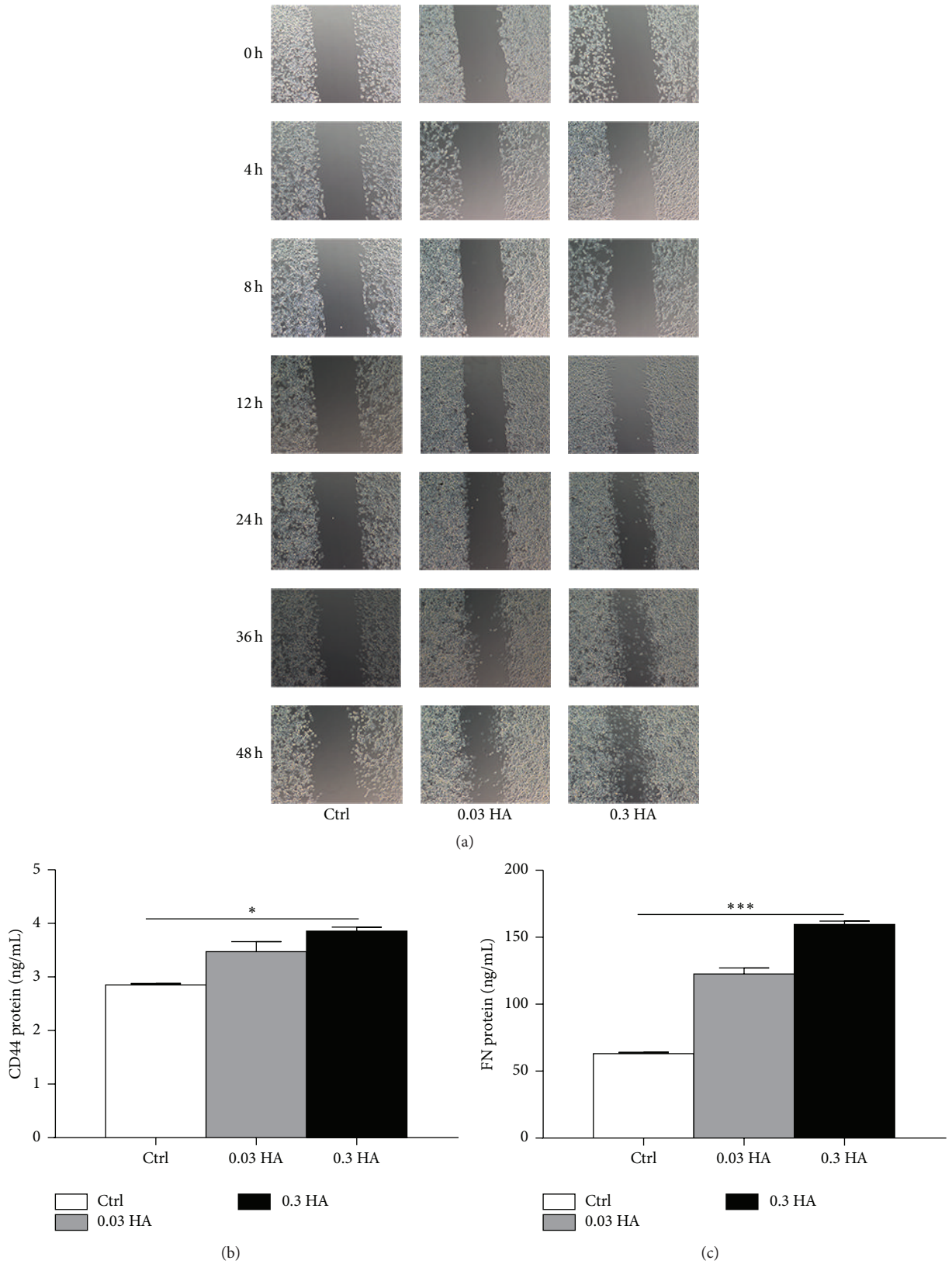

Figure 1: Hyaluronate promoted cell migration and increased the expression of repair factors. Microscopic images (a) of the repair of HCECs in the control, 0.03 HA, and 0.3 HA groups. CD44 (b) and FN (c) protein levels were examined in the control, 0.03 HA, and 0.3 HA groups. Magnification: ×20. The data are the mean ± SEM and represent individual experiments, each including 5 samples/group/time. $^{*}p < 0.05$; $^{***}p < 0.001$.

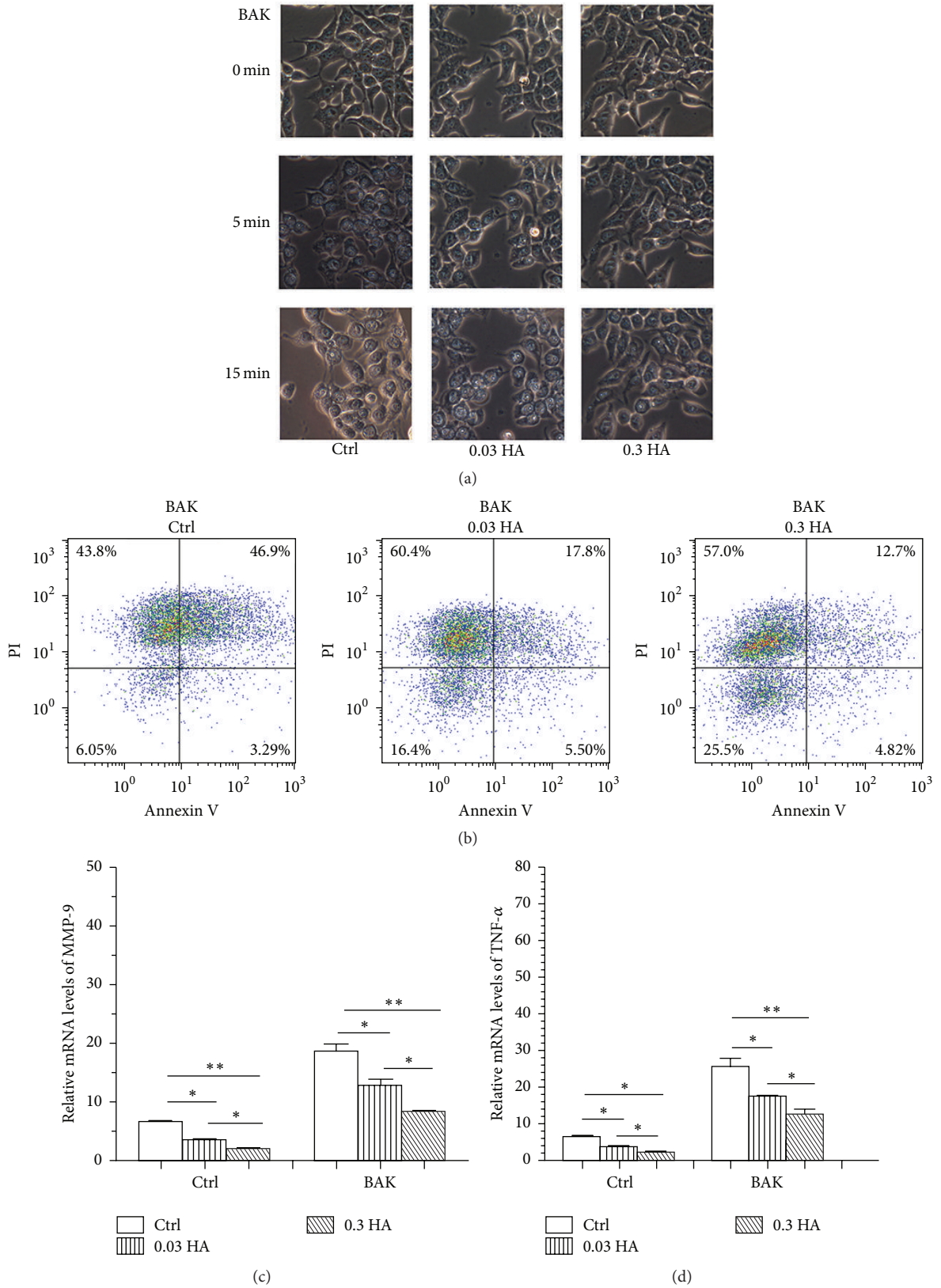

Figure 2: Hyaluronate decreased BAK-induced cell apoptosis and the expression of inflammatory cytokines. Microscopic images (a) of the changes in cell morphology in response to the hyperosmolar conditions were examined in the control, 0.03 HA, and 0.3 HA groups. Flow cytometry (b) was performed to analyse cell apoptosis, and real-time PCR (c) and (d) was used to compare the expression levels of MMP-9 and TNF-α among the three groups. Magnification: ×100. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. The data are the mean ± SEM and represent individual experiments, each including 5 samples/group/time.

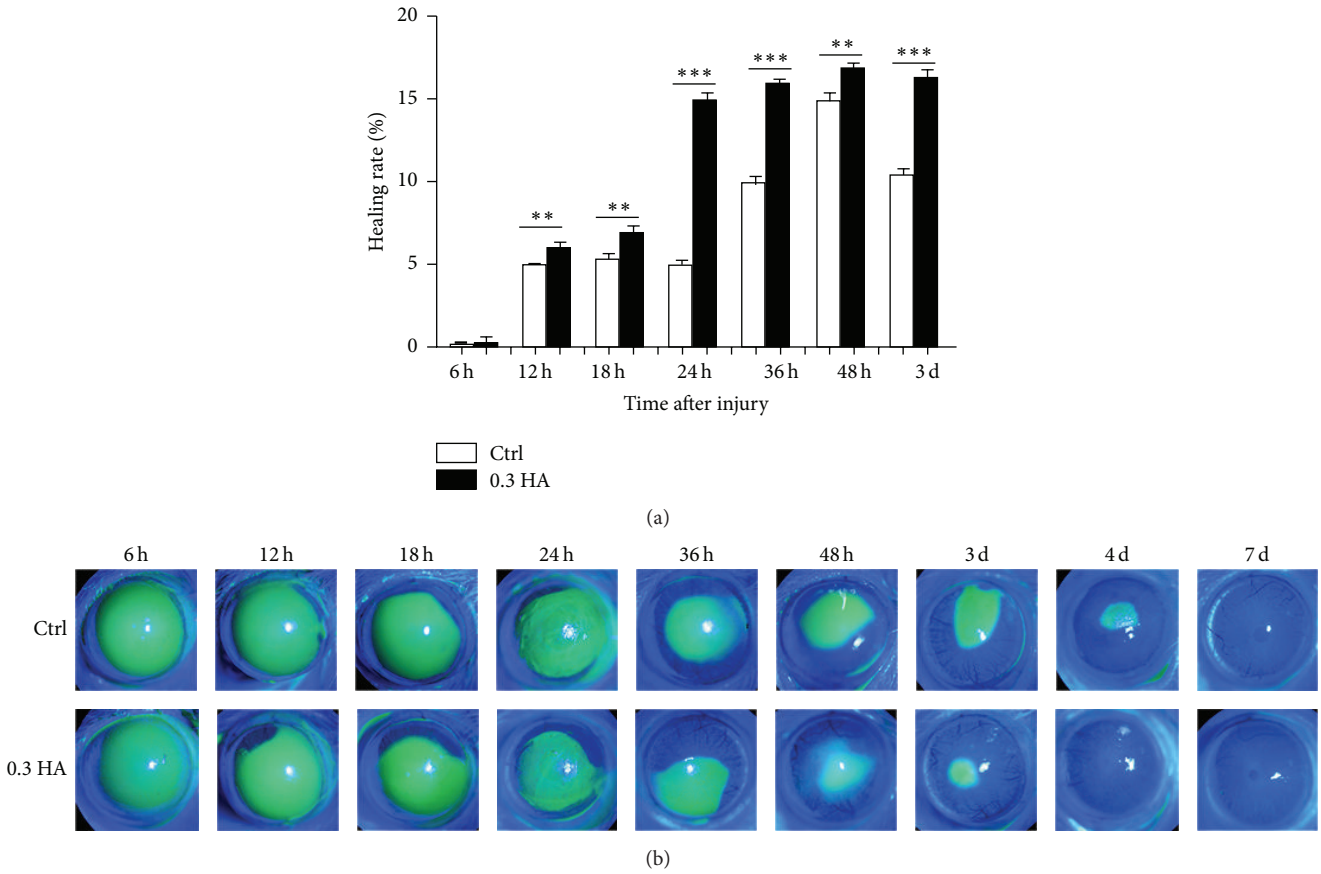

Figure 3: Hyaluronate accelerated corneal epithelial wound healing in the heptanol-burned model. SD rat corneal epithelial cells were removed by burning with heptanol. The healing rates (a) and fluorescein-stained corneal images (b) revealed the healing processes of the corneal lesions in the rats at 6 h, 12 h, 18 h, 24 h, 36 h, 48 h, 3 d, 4 d, and 7 d after injury in the control and 0.3 HA groups. Magnification: ×16, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. The data are the mean ± SEM and represent individual experiments, each including 5 animals/group/time.

enhanced effects. To further explore the role of hyaluronate *in vivo*, we generated an animal model with corneal epithelial defects in which rat corneas were burned with heptanol to damage corneal epithelial cells, and then 0.3% HA eye drops or normal saline was applied to the rats eyes 4 times/day to analyse the reparative effect of hyaluronate on corneal epithelial injury. The healing rates of the control group and the 0.3 HA group are compared in Figure 3(a). These rates were 4.958% in the control group and 6.04% in the 0.3 HA group at 12 h, with peaks of 14.9% in the control group and 17% in the 0.3 HA group at 48 h. The 0.3 HA group exhibited almost complete recovery at 4 d, while recovery was delayed to 7 d in the control group, indicating that the rate was significantly higher in the 0.3 HA group than that in the 0.3 HA group ($p < 0.05$). As shown in Figure 3(b), the rat corneal epithelial cells in the control group migrated gradually after injury and were intact at 7 d, while the 0.3 HA group exhibited almost complete corneal epithelial cell healing at 4 d, which demonstrated that 0.3% HA clearly accelerated the process of corneal epithelial repair. The results clearly demonstrate that hyaluronate promotes the healing of corneal epithelial cells *in vivo*.

3.4. Hyaluronate Modulates the Expression of Inflammatory Cytokines and Repair Factors In Vivo. To explore the mechanism by which hyaluronate promotes the migration of corneal epithelial cells, we examined the expression of select inflammatory cytokines and repair factors by real-time PCR. At 1, 2, and 7 d after injury, the RNA expression levels of the inflammatory cytokines IL-1β and MMP-9 (Figures 4(a) and 4(b)) gradually decreased, and 0.3% HA clearly reduced these levels at all time points (all $p < 0.05$). For example, IL-1β expression was 5 times higher in the control group than in the 0.3 HA group at 1 d after injury, and MMP-9 expression was 2 times higher in the 0.3 HA group at 1 d after injury. Moreover, the repair factors FN and CD44 showed opposite trends as the inflammatory cytokines, exhibiting gradually increased expression after injury (Figures 4(c) and 4(d)). Administration of 0.3% HA significantly increased the expression of FN and CD44. For example, the expression of FN was 4 times higher and the expression of CD44 was 1.5 times higher in the 0.3 HA group than in the control group at 7 d after injury (all $p < 0.05$). These results suggest that 0.3% HA downregulates the expression of inflammatory cytokines and upregulates the expression of repair factors

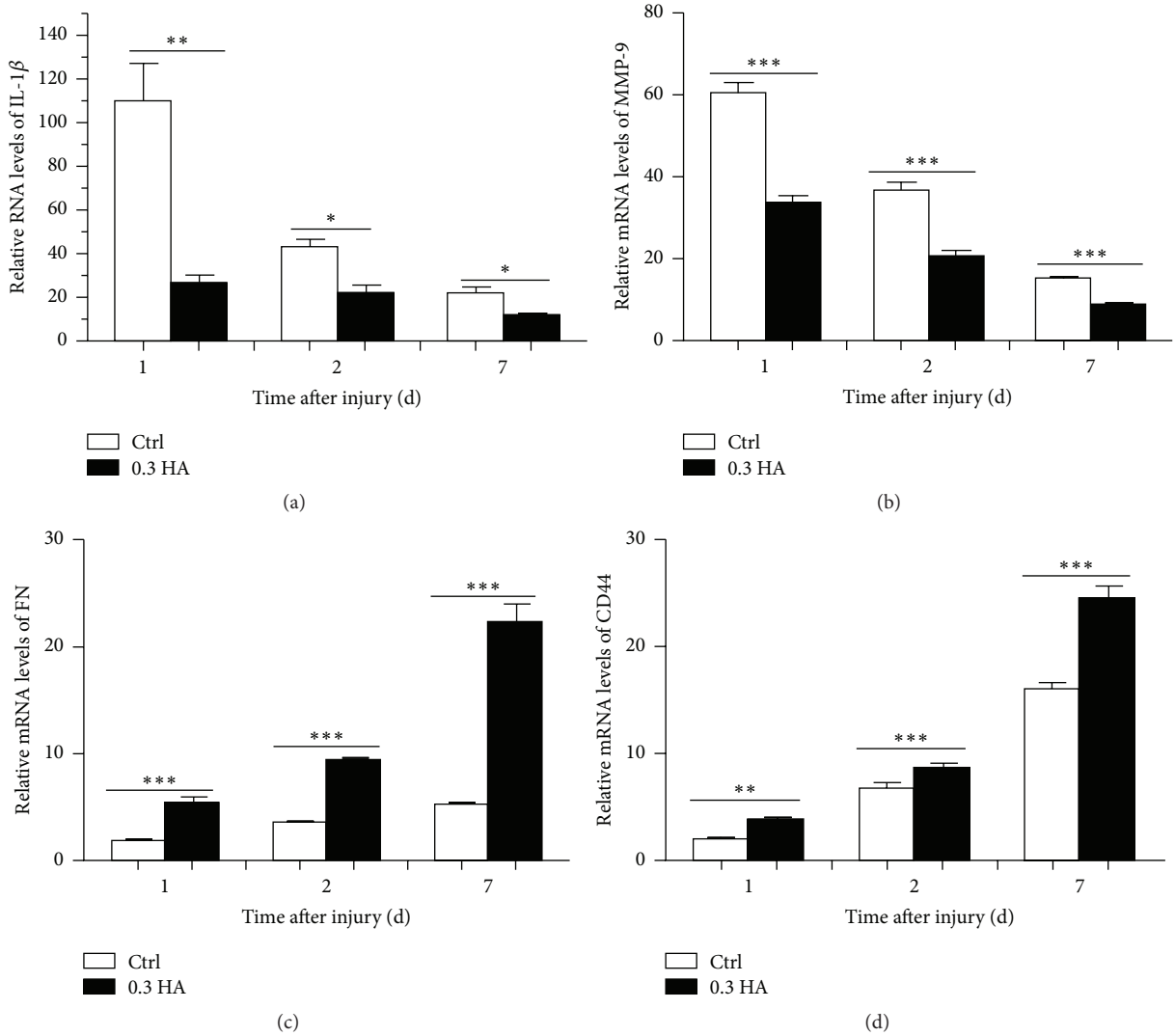

Figure 4: Hyaluronate decreased the expression of inflammatory cytokines and increased the expression of repair factors *in vivo*. The RNA levels of inflammatory cytokines, including IL-1β (a) and MMP-9 (b), and those of repair factors, including FN (c) and CD44 (d), were determined by real-time PCR at 1, 2, and 7 d after injury in the control and 0.3 HA groups. The data are the mean ± SEM and represent individual experiments, each including 5 animals/group/time. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

to attenuate inflammatory responses and improve corneal epithelial healing.

4. Discussion

A healthy corneal epithelium is essential for protecting the eye from infection and structural damage. An intact corneal epithelium is composed of five to seven layers of cells, and its healing process has been reported to include three separate phases [2, 18]. During the first phase, a provisional attachment complex, which is referred to as a focal contact, forms. The epithelial cells flatten and migrate as an intact sheet to cover the wound. During the second phase, cells distal to the original wound proliferate to repopulate the wound area, and cell stratification and differentiation occur. During the third phase, hemidesmosomes form, and extracellular matrix synthesis and reassembly occur [19, 20].

A large number of experimental studies on animals have confirmed that the healing process of corneal epithelial cells involves a complex series of interactions among extracellular matrix proteins, repair factors, and inflammatory cytokines [21–23]. Hyaluronate, an important substance involved in the healing process, has been reported to connect with various extracellular matrix proteins and to efficiently accelerate the healing process [24, 25]. Hyaluronate is found primarily in the extracellular matrix, and its biological functions include maintenance of liquid connective tissue, control of tissue hydration, and water transport. Moreover, the consistency and tissue-friendliness of hyaluronate allow it to serve as a viscosity-enhancing component in eye drops and as an adjuvant for eye tissue repair [26, 27]. However, it remains unclear whether exogenous hyaluronate promotes corneal epithelial cell migration and proliferation similar to endogenous hyaluronate. Additionally, the mechanism by which HA

promotes migration and the dose-effect curve of HA remain unclear.

The process of proliferation is activated rapidly after injury in human corneal epithelial cells, and a large amount of hyaluronate is secreted from the cytoplasm for association with its receptors, such as CD44 and Fibronectin [16, 28]. CD44 is a transmembrane receptor for hyaluronate, and it is also capable of binding Fibronectin, laminin, and collagen I [29]. Fibronectin plays an important role in collagen deposition and stimulates the proliferation and differentiation of corneal epithelial cells and fibroblasts [30]. Evidence suggests that Fibronectin and CD44 play essential roles in providing a transient subepithelial matrix onto which migrating epithelial cells adhere during the frequent cycles of cleavage and attachment of these cells. These cells appear on wound surfaces within an hour after injury and have been found to contain cell receptor sites as well as binding sites for certain basement membrane components, including heparin sulphate and type IV collagen [16, 18, 31]. Once the process of corneal healing is initiated, the expression levels of hyaluronate and its receptors, Fibronectin and CD44, are increased.

Our study has determined the effects of different concentrations of hyaluronate on the migration and proliferation of HCECs *in vitro*, and we have concluded that 0.3% HA results in a higher proliferation rate than 0.03% HA. Therefore, we speculate that the levels of HA and its receptors are positively associated, achieving a "dose-efficiency" effect. Furthermore, the trends of increases in the CD44 and Fibronectin levels indicated that the higher concentration of hyaluronate allowed it to bind to its receptors to more efficiently accelerate the healing process. In addition, we used BAK and NaCl to induce hyperosmolarity and then examined cell changes that occurred under these conditions. BAK is known to be an inducer of oxidative stress and hyperosmolarity, and it can impair protective mechanisms and cause time- and dose-dependent increases in superoxide and ROS levels [32, 33]. We added different concentrations of hyaluronate to assess its protective role against BAK in human corneal epithelial cells. The results revealed that hyaluronate efficiently inhibited BAK-induced injury and delayed cell inactivation and that 0.3% HA exhibited better effects. We deduced that HA reduced the production and activity of proinflammatory mediators and matrix metalloproteinases and that it altered the behaviour of immune cells. These functions were manifested in the scavenging of reactive oxygen-derived free radicals, the inhibition of immune complex adherence to polymorphonuclear cells, the inhibition of leukocyte and macrophage migration and aggregation, and the regulation of fibroblast proliferation [33, 34]. Moreover, apoptosis is a process of programmed cell death that allows for the removal of abnormal cells [35]. Flow cytometry confirmed that hyaluronate efficiently decreased the percentages of late apoptosis and dead cells in agreement with the observation that HA alleviated the morphological changes of cells under adverse conditions.

To further verify the role of HA in promoting and repairing rat corneal epithelial cells, heptanol was used to remove the corneal epithelium to generate a corneal epithelium-injured animal model. The integrity of the corneal epithelial cells was dependent on cell-cell and cell-matrix interactions and on epithelial renewal, which has been widely demonstrated by *in vitro* studies. Considering the function of hyaluronate observed *in vitro* and its hygroscopic property [10], we deduced that it may also be important for modulating tissue hydration, osmotic balance, and cell renewal and differentiation *in vivo*. In our study, the complete repair of injured corneal epithelial cells took 7 days; for the control group, the healing rate was 15.39% at 24 h after injury, and it increased to 40.21% and 50.57% at 48 h and 72 h after injury, respectively. Comparatively, for the 0.3 HA group, the healing rate was 28.48% at 24 h after injury, and it increased to 61.52% and 77.88% at 48 h and 72 h after injury, respectively. Thus, the healing rate in the 0.3 HA group was clearly higher than that in the control group, demonstrating that hyaluronate promoted the repair of corneal epithelial cells.

Moreover, the inflammatory response was activated, and the expression of inflammatory cytokines, including IL-1β and MMP-9, was augmented. A possible relationship between inflammatory cytokines and ocular damage has been reported [36, 37]. In our study, the expression levels of inflammatory cytokines, such as IL-1β and MMP-9, gradually decreased following wounding, while those of the repair cytokines CD44 and FN were increased. It is possible that the proliferation and keratinization of epithelial cells and neovascularization are correlated with the activation of inflammatory cells, initiation of the MAPK signal transduction pathway, and increases in the secretion of inflammatory cytokines [38, 39]. Because hyaluronate promotes the healing of corneal epithelial cells and attenuates the inflammatory response, the protein and RNA levels of inflammatory cytokines, such as IL-1β and MMP-9, were lower in the 0.3 HA group, and those of repair factors, such as CD44 and Fibronectin, were higher compared with the control group.

To summarize, our study has demonstrated that hyaluronate accelerates the proliferation of human corneal epithelial cells and upregulates the expression of repair cytokines, thereby reducing apoptosis and downregulating the expression of inflammatory cytokines *in vitro*; in addition, it efficiently promotes the repair of ocular damage *in vivo*. Overall, this study provides a better understanding of and a promising therapeutic direction for hyaluronate in the treatment of ocular disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Jing Zhong, Yuqing Deng, and Bishan Tian contributed equally to this work.

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References

[1] L. R. Katzman and B. H. Jeng, "Management strategies for persistent epithelial defects of the cornea," *Saudi Journal of Ophthalmology*, vol. 28, no. 3, pp. 168–172, 2014.

[2] N. J. R. Maycock and J. Marshall, "Genomics of corneal wound healing: a review of the literature," *Acta Ophthalmologica*, vol. 92, no. 3, pp. e170–e184, 2014.

[3] K. Lekhanont, P. Jongkhajornpong, L. Choubtum, and V. Chuckpaiwong, "Topical 100% serum eye drops for treating corneal epithelial defect after ocular surgery," *BioMed Research International*, vol. 2013, Article ID 521315, 7 pages, 2013.

[4] H. F. Chen, L. Yeung, K. J. Yang, and C. C. Sun, "Persistent corneal epithelial defect after pars plana vitrectomy," *Retina*, vol. 36, no. 1, pp. 148–155, 2016.

[5] S. Kaštelan, M. Tomić, J. Salopek-Rabatić, and B. Novak, "Diagnostic procedures and management of dry eye," *BioMed Research International*, vol. 2013, Article ID 309723, 6 pages, 2013.

[6] B. Wirostko, M. Rafii, D. A. Sullivan, J. Morelli, and J. Ding, "Novel therapy to treat corneal epithelial defects: a hypothesis with growth hormone," *The Ocular Surface*, vol. 13, no. 3, pp. 204.e1–212.e1, 2015.

[7] M. Moshirfar, K. Pierson, K. Hanamaikai, L. Santiago-Caban, V. Muthappan, and S. F. Passi, "Artificial tears potpourri: a literature review," *Clinical Ophthalmology*, vol. 8, pp. 1419–1433, 2014.

[8] F. Yu, X. Liu, Y. Zhong et al., "Sodium hyaluronate decreases ocular surface toxicity induced by benzalkonium chloride-preserved latanoprost: an in vivo study," *Investigative Ophthalmology & Visual Science*, vol. 54, no. 5, pp. 3385–3393, 2013.

[9] M. J. Rah, "A review of hyaluronan and its ophthalmic applications," *Optometry*, vol. 82, no. 1, pp. 38–43, 2011.

[10] P. N. Sudha and M. H. Rose, "Beneficial effects of hyaluronic acid," *Advances in Food and Nutrition Research*, vol. 72, pp. 137–176, 2014.

[11] V. B. Agrawal and R. J. F. Tsai, "Corneal epithelial wound healing," *Indian Journal of Ophthalmology*, vol. 51, no. 1, pp. 5–15, 2003.

[12] T. Miyazaki, S. Miyauchi, T. Nakamura, S. Takeshita, and K. Horie, "The effect of sodium hyaluronate on the growth of rabbit corneal epithelial cells in vitro," *Journal of Ocular Pharmacology and Therapeutics*, vol. 12, no. 4, pp. 409–415, 1996.

[13] J.-Y. Lai and I.-H. Tu, "Adhesion, phenotypic expression, and biosynthetic capacity of corneal keratocytes on surfaces coated with hyaluronic acid of different molecular weights," *Acta Biomaterialia*, vol. 8, no. 3, pp. 1068–1079, 2012.

[14] W.-T. Ho, T.-H. Chiang, S.-W. Chang, Y.-H. Chen, F.-R. Hu, and I.-J. Wang, "Enhanced corneal wound healing with hyaluronic acid and high-potassium artificial tears," *Clinical & Experimental Optometry*, vol. 96, no. 6, pp. 536–541, 2013.

[15] J. S. Lee, S. U. Lee, C. Y. Che, and J. E. Lee, "Comparison of cytotoxicity and wound healing effect of carboxymethylcellulose and hyaluronic acid on human corneal epithelial cells," *International Journal of Ophthalmology*, vol. 8, no. 2, pp. 215–221, 2015.

[16] J. A. P. Gomes, R. Amankwah, A. Powell-Richards, and H. S. Dua, "Sodium hyaluronate (hyaluronic acid) promotes migration of human corneal epithelial cells in vitro," *British Journal of Ophthalmology*, vol. 88, no. 6, pp. 821–825, 2004.

[17] L. García-Posadas, L. Contreras-Ruiz, A. López-García, S. Villarón Álvarez, M. J. Maldonado, and Y. Diebold, "Hyaluronan receptors in the human ocular surface: a descriptive and comparative study of RHAMM and CD44 in tissues, cell lines and freshly collected samples," *Histochemistry and Cell Biology*, vol. 137, no. 2, pp. 165–176, 2012.

[18] M. A. Stepp, J. D. Zieske, V. Trinkaus-Randall et al., "Wounding the cornea to learn how it heals," *Experimental Eye Research*, vol. 121, pp. 178–193, 2014.

[19] O. Yamanaka, T. Sumioka, and S. Saika, "The role of extracellular matrix in corneal wound healing," *Cornea*, vol. 32, supplement 1, pp. S43–S45, 2013.

[20] C. Chi and V. Trinkaus-Randall, "New insights in wound response and repair of epithelium," *Journal of Cellular Physiology*, vol. 228, no. 5, pp. 925–929, 2013.

[21] I. S. Barequet, N. Harizman, H. Ziv, and M. Rosner, "Healing rate of corneal erosions: comparison of the effect of chloramphenicol eye drops and ointment and high-concentration hyaluronic acid in an animal model," *Cornea*, vol. 33, no. 10, pp. 1080–1082, 2014.

[22] M. Ehrenberg, E. Zolotariov, E. Loeb, V. Poliansky, and A. Levy, "Combining sodium hyaluronate and polyvinylpyrrolidone therapies for the rabbit cornea: a new approach to relief of the human dry eye syndrome," *Current Eye Research*, vol. 40, no. 9, pp. 913–922, 2015.

[23] X. Zheng, T. Goto, and Y. Ohashi, "Comparison of in vivo efficacy of different ocular lubricants in dry eye animal models," *Investigative Ophthalmology & Visual Science*, vol. 55, no. 6, pp. 3454–3460, 2014.

[24] M. Prosdocimi and C. Bevilacqua, "Exogenous hyaluronic acid and wound healing: an updated vision," *Panminerva Medica*, vol. 54, no. 2, pp. 129–135, 2012.

[25] P. L. Bollyky, M. Bogdani, J. B. Bollyky, R. L. Hull, and T. N. Wight, "The role of hyaluronan and the extracellular matrix in islet inflammation and immune regulation," *Current Diabetes Reports*, vol. 12, no. 5, pp. 471–480, 2012.

[26] P. Dahiya and R. Kamal, "Hyaluronic acid: a boon in periodontal therapy," *North American Journal of Medical Sciences*, vol. 5, no. 5, pp. 309–315, 2013.

[27] P. Olczyk, K. Komosińska-Vassev, K. Winsz-Szczotka, K. Kuźnik-Trocha, and K. Olczyk, "Hyaluronan: structure, metabolism, functions, and role in wound healing," *Postepy Higieny I Medycyny Doświadczalnej*, vol. 62, pp. 651–659, 2008.

[28] S. Saika, Y. Ohnishi, A. Ooshima, C.-Y. Liu, and W. W.-Y. Kao, "Epithelial repair: roles of extracellular matrix," *Cornea*, vol. 21, no. 2, supplement 1, pp. S23–S29, 2002.

[29] L. Y. W. Bourguignon, "Matrix hyaluronan-activated CD44 signaling promotes keratinocyte activities and improves abnormal epidermal functions," *The American Journal of Pathology*, vol. 184, no. 7, pp. 1912–1919, 2014.

[30] M. Nakamura and T. Nishida, "Synergistic effects of hyaluronan and fibronectin on epithelial migration in rabbit cornea in vitro," *Cornea*, vol. 18, no. 6, pp. 686–692, 1999.

[31] H. S. Dua, J. A. P. Gomes, and A. Singh, "Corneal epithelial wound healing," *The British Journal of Ophthalmology*, vol. 78, no. 5, pp. 401–408, 1994.

[32] C. Clouzeau, D. Godefroy, L. Riancho, W. Rostène, C. Baudouin, and F. Brignole-Baudouin, "Hyperosmolarity potentiates toxic effects of benzalkonium chloride on conjunctival epithelial cells in vitro," *Molecular Vision*, vol. 18, pp. 851–863, 2012.

[33] H. Wu, H. Zhang, C. Wang et al., "Genoprotective effect of hyaluronic acid against benzalkonium chloride-induced DNA damage in human corneal epithelial cells," *Molecular Vision*, vol. 17, pp. 3364–3370, 2011.

[34] G. D. Burton and R. M. Hill, "Aerobic responses of the cornea to ophthalmic preservatives, measured in vivo," *Investigative Ophthalmology & Visual Science*, vol. 21, no. 6, pp. 842–845, 1981.

[35] S. E. Wilson, "Role of apoptosis in wound healing in the cornea," *Cornea*, vol. 19, supplement 3, pp. S7–S12, 2000.

[36] M. Calonge, A. Enríquez-de-Salamanca, Y. Diebold et al., "Dry eye disease as an inflammatory disorder," *Ocular Immunology and Inflammation*, vol. 18, no. 4, pp. 244–253, 2010.

[37] M. Ueta and S. Kinoshita, "Innate immunity of the ocular surface," *Brain Research Bulletin*, vol. 81, no. 2-3, pp. 219–228, 2010.

[38] J. Hong, T. Qian, Q. Le et al., "NGF promotes cell cycle progression by regulating D-type cyclins via PI3K/Akt and MAPK/Erk activation in human corneal epithelial cells," *Molecular Vision*, vol. 18, pp. 758–764, 2012.

[39] K. Terai, M. K. Call, H. Liu et al., "Crosstalk between TGF-beta and MAPK signaling during corneal wound healing," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 11, pp. 8208–8215, 2011.

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Clinical Study

Repeatability and Reproducibility of Noninvasive Keratograph 5M Measurements in Patients with Dry Eye Disease

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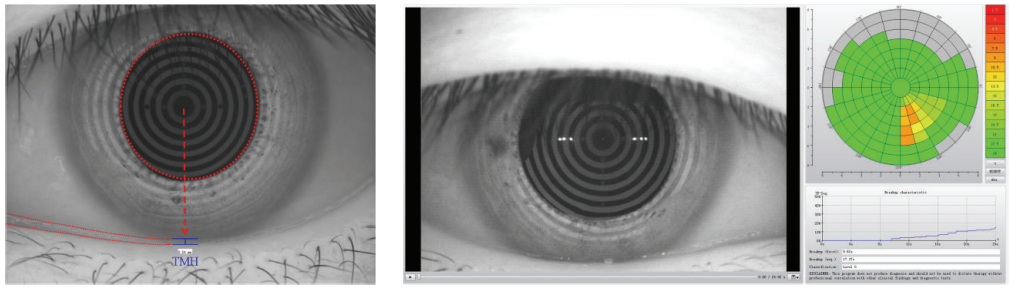
Purpose. To determine the intraexaminer repeatability and interexaminer reproducibility of tear meniscus height (TMH) and noninvasive Keratograph tear breakup time (NIKBUT) measurements obtained with the Keratograph 5M (K5M) in a sample of healthy and dry eye populations. *Methods*. Forty-two patients with dry eye disease (DED group) and 42 healthy subjects (healthy group) were recruited in this prospective study. In all subjects, each eye received 3 consecutive measurements using the K5M for the TMH and NIKBUTs (NIKBUT-first and NIKBUT-average). And then a different examiner repeated the measurements. The repeatability and reproducibility of measurements were assessed by the coefficient of variation (CV) and intraclass correlation coefficient (ICC). *Results*. The repeatability and reproducibility of TMH and NIKBUTs were good in both DED and healthy groups (CV% $\leq$ 26.1% and ICC $\geq$ 0.75 for all measurements). Patients with DED showed better intraexaminer repeatability for NIKBUTs, but worse for TMH than healthy subjects. Average TMH, NIKBUT-first, and NIKBUT-average were significantly lower in DED group than in healthy group (all P values < 0.05). *Conclusions*. Measurements of TMH and NIKBUTs obtained with the K5M may provide a simple, noninvasive screening test for dry eye with acceptable repeatability and reproducibility. The NIKBUTs were more reliable, but TMH was less reliable in patients with DED.

1. Introduction

Dry eye disease (DED) is a chronic, multifactorial disease of the tears and ocular surface, which is caused by either decreased tear production or increased tear film evaporation [1]. DED is one of the most common ocular disorders, with symptoms affecting 5–30% of the population worldwide; however in many cases it is underdiagnosed and left undertreated [2]. The cornea is the transparent front part of the eye and the tear film ensures a smooth refracting surface and prevents microbial invasion [3]. As a result, the instability of a disrupted tear film over the irregular surface of a dry eye is thought to affect the quality of vision [4]. Many attempts have been made to define the characteristics of dry eye; however, no "gold standard" exists till now. Traditionally, common objective clinical measures assessing the tear film and diagnosing DED are known as the fluorescein tear breakup time (FBUT) and Schirmer test [5, 6]. But the traditional objective tests are often limited by their invasiveness and low test repeatability and reproducibility [7, 8].

The tear meniscus refers to the tears lying. It has been estimated that 75–90% of tear volume is accounted for by the tear meniscus [9]. Some previous studies reported that a positive correlation between the tear meniscus height (TMH) and Schirmer test value has been found [10, 11]. So the TMH can be considered as a noninvasive test for the quantitative of tears [12].

Recent advanced Placido topograph, the Keratograph 5M (K5M; Oculus Optikgeräte GmbH, Wetzlar, Germany), has additional imaging modalities designed to noninvasively measure TMH and noninvasive Keratograph tear breakup time (NIKBUT) [13–15]. And it has been used in the evaluation of tear film and diagnosis of DED [12]. In this study, the intraexaminer repeatability and interexaminer reproducibility of the measurements for TMH and NIKBUTs were evaluated, and their results in the DED patients and the healthy population were compared.



(a) (b)

Figure 1: The representative outputs of TMH and NIKBUT. (a) The TMH was measured perpendicular to the lid margin at the central point relative to the pupil center. (b) NIKBUT result map included the color-coded map, breakup characteristics map, and first and average breakup time and classification.

2. Methods

2.1. Subject Recruitment. This prospective study involved 84 eyes of 84 subjects: 42 eyes with DED not associated with Sjögren's syndrome (DED group) and 42 healthy control eyes (healthy group). In patients who were diagnosed with DED in only one eye, that eye was selected for measurement. For participants with DED in both eyes and for healthy subjects, right eye was selected for measurement and statistical analysis. The diagnosis of dry eye was made according to the consensus of DED in China (2013): (1) at least 1 of 6 symptoms: dryness, burning, sandiness, tiredness, discomfort, and blurred vision with FBUT ≤5 s or a nonanesthesia Schirmer I test value ≤5 mm/5 min; (2) at least 1 of 6 symptoms: dryness, burning, sandiness, tiredness, discomfort, and blurred vision with 5 s < FBUT ≤ 10 s or 5 mm/5 min < nonanesthesia Schirmer I test ≤ 10 mm/5 min, accompanied by corneal fluorescein staining score. Exclusion criteria for both groups were as follows: age <18 years, subject unable to complete the questionnaire or understand the procedures, the presence of ocular or systemic disease or the use of topical or systemic medications that may affect the cornea and the ocular surface (except the use of nonpreserved tear substitutes in the DED group), and previous eye surgery or contact lens wore in the past 24 hr.

Data were collected from July to December 2015 in Beijing Tongren Hospital, Beijing, China. All participants signed an informed consent form in accordance with the tenets of the Declaration of Helsinki and this study was approved by the institutional review board of Beijing Tongren Hospital, Beijing, China.

2.2. Ocular Examinations. Each patient was asked to complete the Ocular Surface Disease Index (OSDI) questionnaire (range: 0–100). In all eyes, ophthalmic examination was performed in the same order as follows: firstly, TMH measurement and then NIKBUTs measurement with Keratograph 5M, FBUT assessment, corneal and conjunctival fluorescein staining, nonanesthetized Schirmer I test, and corneal sensation measured with the Cochet-Bonnet esthesiometer (Luneau, Prunay-Le-Gillon, France).

2.3. Keratograph 5M Measurement. All subjects underwent imaging with the K5M equipped with a modified tear film scanning function. In each subject, inferior TMH images were captured and measured perpendicular to the lid margin at the central point relative to the pupil center using an integrated ruler. The principle and technique for NIKBUT measurements have been described previously [12, 16]. NIKBUT was measured as the time in seconds between the last complete blink and the first perturbation of placid rings projected onto the surface of the cornea, which the device automatically detects. K5M generated two measures for NIKBUT: the time at the first breakup of tear film occurs (NIKBUT-first) and the average time of all breakup incidents (NIKBUT-average). The representative outputs for TMH and NIKBUT were shown in Figure 1.

2.4. Fluorescein Tear Film Breakup Time and Corneal Staining Score. Fluorescein dye was used to assess corneal staining and FBUT. A sterile fluorescein strip moistened with ocular irrigation solution was applied to the inferior fornix. Two or three minutes later, the subjects were requested to blink several times to ensure adequate mixing of the dye and then keep their eyes open. FBUT was examined under standard illumination using a slit-lamp microscope with a cobalt-blue filter, and the time was recorded with a stopwatch. FBUT is the time interval between the last blink and the appearance of the first random dry spot on the corneal surface. The average of three consecutive FBUT values was calculated. Corneal and conjunctival staining was evaluated under a yellow filter using the Oxford scale and after instillation of fluorescein.

2.5. Schirmer I Test. Schirmer I test was a useful assessment of aqueous tear production. The inferior conjunctival fornix was dried with a cotton stick. One minute later, a standard 5 × 40 mm Schirmer test strip was placed over the junction of the middle and outer third of inferior lid. The patients are instructed to keep their eyes closed during the test. The test lasted 5 minutes, and the amount of wetting was recorded.

2.6. Repeatability and Reproducibility of the TMH and NIBUT Measurements. To measure the intraexaminer repeatability, the TMH and NIBUT were calculated using 3 consecutive measurements by the same masked clinician. To measure interexaminer reproducibility, the participants were tested

Table 1: Characteristics of the study population.

Parameters	Healthy ($n = 42$)	Dry eye disease ($n = 42$)	P value
Age (year)	38.76 ± 13.18	41.43 ± 15.77	0.403
Gender (male/female)	12/30	14/28	0.637
OSDI (score)	3.74 ± 6.90	30.37 ± 15.11	<0.001
FBUT (s)	9.15 ± 3.51	4.59 ± 1.71	<0.001
Schirmer test (mm/5 min)	15.48 ± 8.68	8.21 ± 5.68	<0.001
Oxford scale	0.00 ± 0.00	1.16 ± 1.54	<0.001
Corneal sensation (mm)	6.07 ± 0.09	5.63 ± 0.47	<0.001
TMH (mm)	0.27 ± 0.12	0.22 ± 0.07	0.02
NIKBUT-first (s)	7.36 ± 3.99	5.57 ± 3.31	0.028
NIKBUT-average (s)	10.35 ± 4.22	8.08 ± 4.08	0.014

FBUT = fluorescein tear breakup time; NIKBUT = noninvasive Kertograph tear breakup time; TMH = tear meniscus height; OSDI = Ocular Surface Disease Index.

by 2 independent and well-trained clinicians in random order, and the agreement between them was analyzed. The participants were given a 10-minute pause between each measurement. All the evaluators were masked to the subjects' clinical and demographic details. All the measurements were taken between 10:00 a.m. and 16:00 p.m. in one day and in a dimly lit room where the temperature (20–25°C) and humidity (30–40%) were controlled.

2.7. Statistical Analysis. Two software programs, SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA) and MedCalc 13.0 (MedCalc Software, Ostend, Belgium), were used to conduct the statistical analyses. Data were test for normality using the Kolmogorov-Smirnov test, which were here provided as the mean and standard deviation (SD). Differences between groups (DED and healthy) were evaluated using the Welch modified Student two-sample t-test and the Wilcoxon rank-sum test. A χ^2 test was performed for gender distribution. To assess intraexaminer repeatability and interexaminer reproducibility, the within-subject SD (S_w), precision ($1.96S_w$), repeatability ($2.77S_w$), and coefficient of variation (CV) were calculated from the 3 consecutive K5M measurements [17]. The intraclass correlation coefficient (ICC) was also applied for the interexaminer repeatability (ICC ≥ 0.75 indicated good reliability) [18]. All P values were 2-sided and considered as statistically significant when <0.05.

3. Results

3.1. Demographics. A total of 42 dry eye patients and 42 healthy subjects were recruited for the study. Table 1 showed that there was no significant difference in age and gender distribution between the two groups. The OSDI and Oxford scale values were significantly less, while FBUT, Schirmer test, and corneal sensation values were significantly more for the DED group than for the healthy group. The TMH and NIKBUTs values were also significantly lower in the DED group.

3.2. Intraexaminer Repeatability and Interexaminer Reproducibility. Table 2 showed the mean values, precision, repeatability, CV%, ICC, and 95% confidence interval of the TMH, NIKBUT-first, and NIKBUT-average for the 3 consecutive repeated measurements in DED and healthy groups. The CV% values were within 26.1%, and the ICCs were more than 0.75 for all parameters. Thus, the intraexaminer repeatability of TMH and NIKBUTs measurements by the K5M was good.

Table 2 also showed the mean values, precision, repeatability, and CV% of the TMH, NIKBUT-first, and NIKBUT-average for the interexaminer reproducibility. The CV% values were within 21.85%, and the precision values were within 3.94 and the repeatability values were within 5.14. These also indicated good interexaminer reproducibility.

4. Discussion

The tear film is essential for maintaining the health of the ocular surface and also it is an important optical element, which ensures a smooth refracting surface [19]. It forms a complex and stable system in ocular surface. As a result, the instability of a disrupted tear film may compromise ocular health and lead to dry eye. In clinical practice, FBUT is the most widely performed examination to aid in assessing the tear film stability. Although FBUT measurement using fluorescein dye is a minimally invasive technique, fluorescein instillation can destabilize the tear film [7]. The Schirmer test, on the other hand, is the most commonly used test to measure tear production, which is an indispensable component of examination in patients with DED. But it has been suggested to have low reproducibility, with wide variations occurring between subjects and on different days/visits, and the reliability of the test can be affected by environmental conditions, for example, temperature and humidity [8, 20]. Non- or minimally invasive dry eye tests have the major advantage without significantly inducing reflex tearing, which can subsequently affect results following the invasive procedure. These types of noninvasive techniques, such as K5M, have the potential to represent the "true" state of the ocular surface [5]. In the current study, the TMH and NIKBUTs were measured using K5M in patients with DED and healthy subjects. To the best of our knowledge, this is the first study to compare the repeatability and reproducibility of TMH and NIKBUTs measured by K5M in patients with DED. The results of

Table 2: Intraexaminer repeatability and interexaminer reproducibility of TMH and NIKBUTs in Healthy and DED groups.

Parameters	Healthy ($n = 42$)						Dry eye disease ($n = 42$)					
	Mean ± SD	Precision	Repeatability	CV%	ICC	95% CI for ICC	Mean ± SD	Precision	Repeatability	CV%	ICC	95% CI for ICC
Intraexaminer repeatability												
TMH (mm)	0.26 ± 0.12	0.10	0.14	18.89	0.84	0.75 to 0.90	0.24 ± 0.08	0.08	0.11	15.96	0.76	0.64 to 0.85
NIKBUT first (s)	7.25 ± 3.45	3.70	5.24	26.10	0.75	0.62 to 0.84	5.77 ± 3.04	2.67	3.76	23.54	0.82	0.73 to 0.89
NIKBUT average (s)	10.61 ± 3.93	3.96	5.60	19.06	0.78	0.66 to 0.86	8.27 ± 3.86	3.04	4.29	18.71	0.85	0.77 to 0.91
Interexaminer reproducibility												
TMH (mm)	0.26 ± 0.15	0.10	0.14	19.76	—	—	0.24 ± 0.08	0.08	0.11	16.08	—	—
NIKBUT first (s)	7.35 ± 3.68	2.92	4.13	20.30	—	—	5.87 ± 3.07	2.51	3.55	21.85	—	—
NIKBUT average (s)	10.71 ± 4.11	3.65	5.15	17.37	—	—	8.21 ± 3.93	2.78	3.94	17.30	—	—

SD = standard deviation; TMH = tear meniscus height; NIKBUT = noninvasive Keratograph tear breakup time; CV = coefficient of variation; ICC = intraclass correlation coefficient; 95% CI = 95% confidence interval for the mean.

this study reveal the good repeatability and reproducibility of TMH and NIKBUTs measurements. Patients with DED exhibited lower TMH and shorter NIKBUTs than healthy subjects.

"Repeatability" is defined as the variability in repeated measures by one examiner without changing all other factors. "Reproducibility" refers to the variability in repeated measures when factors are varied [21]. The importance of longitudinal observation of clinical findings in diagnosis and treatment emphasizes the importance of repeatability of its measurements and assesses the reproducibility of its readings with different examiners, when a new instrument is used in clinical practice. Previous repeatability studies of Oculus Keratograph systems have been conducted predominantly on healthy subjects [22], but the repeatability and reproducibility measures in patients with DED were reported rarely; therefore, understanding of the performance of K5M test in a dry eye sample is largely unknown.

Previous studies have evaluated repeatability of NIKBUTs in healthy subjects and have reported results ranging from good reliability [16] to poor reliability [22]. Consistent with the 95% limits of agreement, the ICCs for the NIKBUTs were good in the current study. This study found that the NIKBUTs were more reliable tests in DED group than in healthy group in producing less varied results and more repeatability. Differences in the measurements can be attributed not only to the instrument and operator but also to changes that occur in the eye. According to reduced corneal sensitivity reported in dry eye patient populations, the influence of reflex tearing was less in DED eyes than in healthy eyes [12]. These may explain, in part, why the reliabilities of NIKBUTs were higher in DED group in this study. The relationship between tear function or stability and corneal sensitivity in DED is of interest and should be clarified in future studies. Although the corneal epithelial abnormalities, which presented as corneal staining, may influence the result of repeatability and reproducibility measurements, there were a few eyes showing staining in the DED group, so they can be ignored.

The reliability for measuring TMH already had been established in healthy population with a good intraexaminer repeatability (CV% = 0.16% and ICC = 0.83, resp.) [14], but until now there is no data in patients with DED. Our results showed that the repeatability and reproducibility of TMH reached a good level in DED group, but the TMH was less reliable than healthy subjects. K5M also has its shortcomings: the eyelid margin or the upper margin of the lower meniscus cannot be delineated automatically and the image obtained with the K5M was poor, which made it difficult to correctly delineate the tear meniscus. All of these might compromise the repeatability and reproducibility of its measures.

On the basis of the measurement repeatability and reproducibility, the NIKBUT-first and NIKBUT-average in DED group were significantly shorter than those in healthy group in this study (Table 1). Koh et al. [23] report NIKBUT-first values of 9.71 ± 6.68 s for the healthy eyes and 4.59 ± 1.25 s for the dry eyes. Our results of the NIKBUT-first values were consistent with Koh et al.'s finding, whereas the NIKBUT-first values obtained in Hong et al.'s study [16] (4.3 ± 0.3 s for the healthy eyes and 2.0 ± 0.2 s for the dry eyes) were shorter than the results of the current study. These differences may be explained, in part, by differences in the version of the software by Oculus. The software version was Keratograph 4 in Hong et al.'s study, while in Koh et al.'s and our study, the software was Keratograph 5M.

Using the K5M, TMH was imaged and easily quantified in both the healthy and DED groups. Previous studies [10, 11, 24], using optical coherence tomography, have found it to be significantly decreased in TMH values of dry eyes compared with those of healthy eyes. In the current study, the mean TMH values were 0.22 ± 0.07 mm for DED group and 0.27 ± 0.12 for healthy group. Correspondingly, Hong et al. [16] compared dry eye patients with healthy controls and reported similar lower values for TMH (0.269 ± 0.011 versus 0.379 ± 0.015 mm, resp.) measured by Keratograph 4. Koh et al. [12] also reported that the TMH values were 0.14±0.03 and 0.20 ± 0.05 mm in patients with DED and healthy subjects, respectively. It was shown that our results were somewhere between the results of those two studies. These differences may be explained, in part, by differences in diagnostic criteria for dry eye and in different age stages. Moreover, the poor resolution of TMH images made it difficult to correctly delineate the tear meniscus, especially in the DED group with lower TMH. This might eventually cause the measurement deviation with different examiners.

Age is an important risk factor for DED, and age has been shown to affect the TMH values, with tear menisci in general decreasing with age [24, 25]. The mean ages of the groups in the current study were close to each other, and there was no statistically significant difference between the groups ($P = 0.403$). Therefore, a difference in age is not the reason for the observed differences in the TMH of the groups.

However, the current study had a few limitations. This was an observational cross-sectional study. It is not possible to determine how the longitudinal change of DED progression is related to the TMH and NIKBUTs. The sample size of this study was relatively small and therefore the results should be interpreted cautiously. The intersession repeatability, which is a test in a different day with the same examiner, was not included in this study. Further study with long-term follow-up, larger sample size, and intersession repeatability test is required to explore our findings and the findings of others in greater detail.

In conclusion, noninvasive ocular surface examinations using K5M showed differences in the TMH and NIKBUTs in DED and healthy groups. And K5M may provide a simple, noninvasive screening test for dry eye with acceptable repeatability and reproducibility. It should be considered as an alternative method in the diagnosis and follow-up of patients with DED. Whether its results are more dependable than those obtained with the Schirmer test and FBUT needs further evaluation in studies with a larger patient population.

Disclosure

This paper has not been previously published by the authors. All authors concur with the submission.

Competing Interests

The authors have neither conflict of interests nor commercial interests in the devices mentioned in the paper. No conflicting relationship exists for any author.

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References

[1] M. A. Lemp and G. N. Foulks, "The definition and classification of dry eye disease," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[2] "The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 93–107, 2007.

[3] R. J. Braun, P. E. King-Smith, C. G. Begley, L. Li, and N. R. Gewecke, "Dynamics and function of the tear film in relation to the blink cycle," *Progress in Retinal and Eye Research*, vol. 45, pp. 132–164, 2015.

[4] D. H. Szczesna, J. Jaroński, H. T. Kasprzak, and U. Stenevi, "Interferometric measurements of dynamic changes of tear film," *Journal of Biomedical Optics*, vol. 11, no. 3, Article ID 034028, 2006.

[5] A. J. Bron, J. A. Smith, and M. Calonge, "Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 108–152, 2007.

[6] S. P. Phadatare, M. Momin, P. Nighojkar, S. Askarkar, and K. K. Singh, "A comprehensive review on dry eye disease: diagnosis, medical management, recent developments, and future challenges," *Advances in Pharmaceutics*, vol. 2015, Article ID 704946, 12 pages, 2015.

[7] L. S. Mengher, A. J. Bron, S. R. Tonge, and D. J. Gilbert, "A non-invasive instrument for clinical assessment of the pre-corneal tear film stability," *Current Eye Research*, vol. 4, no. 1, pp. 1–7, 1985.

[8] K. K. Nichols, G. L. Mitchell, and K. Zadnik, "The repeatability of clinical measurements of dry eye," *Cornea*, vol. 23, no. 3, pp. 272–285, 2004.

[9] F. J. Holly, "Physical chemistry of the normal and disordered tear film," *Transactions of the Ophthalmological Societies of the United Kingdom*, vol. 104, no. 4, pp. 374–380, 1985.

[10] O. M. A. Ibrahim, M. Dogru, Y. Takano et al., "Application of visante optical coherence tomography tear meniscus height measurement in the diagnosis of dry eye disease," *Ophthalmology*, vol. 117, no. 10, pp. 1923–1929, 2010.

[11] R. Altan-Yaycioglu, S. Sizmaz, H. Canan, and M. Coban-Karatas, "Optical coherence tomography for measuring the tear film meniscus: correlation with schirmer test and tear-film breakup time," *Current Eye Research*, vol. 38, no. 7, pp. 736–742, 2013.

[12] S. Koh, C. Ikeda, S. Watanabe et al., "Effect of non-invasive tear stability assessment on tear meniscus height," *Acta Ophthalmologica*, vol. 93, no. 2, pp. e135–e139, 2015.

[13] N. S. Abdelfattah, A. Dastiridou, S. R. Sadda, and O. L. Lee, "Noninvasive imaging of tear film dynamics in eyes with ocular surface disease," *Cornea*, vol. 34, pp. S48–S52, 2015.

[14] P. Arriola-Villalobos, J. I. Fernández-Vigo, D. Díaz-Valle, J. E. Peraza-Nieves, C. Fernández-Pérez, and J. M. Benítez-Del-Castillo, "Assessment of lower tear meniscus measurements obtained with Keratograph and agreement with Fourier-domain optical-coherence tomography," *British Journal of Ophthalmology*, vol. 99, no. 8, pp. 1120–1125, 2015.

[15] X. Wang, X. Lu, J. Yang et al., "Evaluation of dry eye and meibomian gland dysfunction in teenagers with myopia through noninvasive keratograph," *Journal of Ophthalmology*, vol. 2016, Article ID 6761206, 5 pages, 2016.

[16] J. Hong, X. Sun, A. Wei et al., "Assessment of tear film stability in dry eye with a newly developed keratograph," *Cornea*, vol. 32, no. 5, pp. 716–721, 2013.

[17] J. M. Bland and D. G. Altman, "Measurement error," *British Medical Journal*, vol. 312, no. 7047, p. 1654, 1996.

[18] A. G. Oliveira, *Biostatistics Decoded*, John Wiley & Sons, New York, NY, USA, 2013.

[19] A. Ribelles, C. Galbis-Estrada, M. A. Parras, B. Vivar-Llopis, C. Marco-Ramírez, and M. Diaz-Llopis, "Ocular surface and tear film changes in older women working with computers," *BioMed Research International*, vol. 2015, Article ID 467039, 10 pages, 2015.

[20] G. Savini, P. Prabhawasat, T. Kojima, M. Grueterich, E. Espana, and E. Goto, "The challenge of dry eye diagnosis," *Clinical Ophthalmology*, vol. 2, no. 1, pp. 31–55, 2008.

[21] C. McAlinden, J. Khadka, and K. Pesudovs, "Statistical methods for conducting agreement (comparison of clinical tests) and precision (repeatability or reproducibility) studies in optometry and ophthalmology," *Ophthalmic and Physiological Optics*, vol. 31, no. 4, pp. 330–338, 2011.

[22] S. M. Cox, K. K. Nichols, and J. J. Nichols, "Agreement between automated and traditional measures of tear film breakup," *Optometry & Vision Science*, vol. 92, no. 9, pp. e257–e263, 2015.

[23] S. Koh, C. Ikeda, H. Fujimoto et al., "Regional differences in tear film stabilityand meibomian glands in patients with aqueous-deficient dry eye," *Eye and Contact Lens*, 2015.

[24] X. Qiu, L. Gong, X. Sun, and H. Jin, "Age-related variations of human tear meniscus and diagnosis of dry eye with fourier-domain anterior segment optical coherence tomography," *Cornea*, vol. 30, no. 5, pp. 543–549, 2011.

[25] L. Cui, M. Shen, J. Wang et al., "Age-related changes in tear menisci imaged by optical coherence tomography," *Optometry & Vision Science*, vol. 88, no. 10, pp. 1214–1219, 2011.

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Clinical Study

Influence of Septal Deviation on the Prognosis of Transcanalicular Diode Laser-Assisted Dacryocystorhinostomy

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Purpose. The objective of the present study is to determine whether the success rate in transcanalicular diode laser-assisted dacryocystorhinostomy (TCL DCR) is influenced by the variant septal deviation (SD). *Methods.* Patients were divided into two groups: one including operated lacrimal pathways (LP) with no anatomical nasosinusal variants and the other group of LP with SD. This study began on January 1, 2008, and ended on December 31, 2010, at Morales Meseguer Hospital. Variables were compared by means of ANOVA and a logistic regression model (LOGIT). *Results.* Out of the 159 LP operated on, 102 had no nasosinusal anatomic variant, but 39 LP were associated with SD. The first group evidenced a success rate of 67.64%, while the second group evidenced a success rate of 66.7%. *Conclusion.* We found no significant statistical differences between the success rates in the two groups (with SD and no anatomical variants). So we could avoid previous or concomitant septoplasty in some cases (mild and moderate SD).

1. Introduction

When a septal deviation (SD) is present during the endoscopy performed before a transcanalicular diode laser-assisted dacryocystorhinostomy (TCL DCR), one tends to think that this anatomical alteration could influence the final result, due to technical difficulties and alterations in osteotomy healing.

Dacryocystorhinostomy (DCR) is the surgical treatment for opening up lacrimal pathways [1, 2]. There are three types of DCRs: external, endoscopic, and transcanalicular approach [3–6].

We use the transcanalicular approach (TCL DCR) for all patients, except recurrences, which we treat externally.

Endoscopically nasosinusal anatomical variations may appear, such as septal deviation (SD), inferior turbinate hypertrophy, and concha bullosa [7–9]. SD seems to be the most frequent nasosinusal anatomic variant. Some patients have no symptoms in spite of SD [7].

We designed the present study in order to investigate the influence of nasal septal deviation on the surgery of lacrimal pathways. If significant influence cannot be proved, then previous or concomitant septoplasty could be avoided in candidates for surgery of the lacrimal pathways (LP).

2. Materials and Methods

From January 1, 2008, until December 31, 2010, one hundred and twenty-four patients were considered candidates for TCL DCR surgery, so they were included in this study.

A protocol was designed, including personal data, medical history, and degree of epiphora. In order to quantify and standardize the degree of epiphora more precisely, we used the Munk score [10]. Candidates for TCL DCR surgery had to meet the following conditions: need to dry tears more than 5 times a day (Munk score: 3–5), blockage of the

vertical part of the lacrimal pathways, presence of lacrimal sac proved by dacryocystography, symptoms (epiphora), chronic dacryocystitis, and/or a history of acute episodes.

The patients were seen by the otorhinolaryngologist and the ophthalmologist in the same follow-up visit, in order to assess the feasibility of TCL DCR; septal deviations were noted. During this multidisciplinary consultation, the ENT specialist wrote down type and degree of SD. There are various classifications of SD [7, 11, 12]. We chose a classification based on the distance separating septum from lateral nasal wall [12]: mild (less than 50% the distance from septum to nasal wall); moderate (greater than 50% the said distance); and severe (septum touched the lateral nasal wall).

Patients younger than eighteen, with previous DCR (whatever the technique), with other disorders of ocular annexes, or lacking in motivation (they interfere with the Munk score) were excluded on this study.

All patients signed the consent form for TCL DCR to participate in this clinical trial and were operated on without taking into consideration the presence of SD or the lack of it.

This study was successfully approved by the ethics committee at Morales Meseguer Hospital.

A Varius laser was used during the surgery. This device uses an InGaAsP diode laser generator and a semiconductor with a wavelength of 980 nm (±5) with a maximum input power of 20 W. The corresponding silica optical fiber was sterile and disposable, measuring 600 microns. The ENT used a Karl Storz tube with an optical angle of 0 degrees.

During the postoperative period topical treatment was prescribed, with tobramycin and dexamethasone eye drops. Twenty-four hours after surgery nasal irrigation with saline water and topical nasal applications of fluticasone furoate were prescribed.

During the follow-up revisions endoscopies (one month, three months, and six months) were performed and traces of fibrin were removed; an assessment was also carried out, taking into account presence of epiphora (Munk scale), positive or negative nasal syringing with fluids, and endoscopic appearance of the osteotomy site.

Surgery was deemed "success" when the patients scored 0 or 1 on the Munk scale, in which they had to dry tears twice or less than twice a day, six months after the operation.

Therefore, we considered "failure" when they scored 2 to 5 on the Munk scale.

We divided the patients who had undergone TCL DCR into two groups: one group included LP with no anatomical nasosinusal variants and the other LP group was with SD or other nasosinusal alterations. Finally, we calculated the success rate for each group.

This prospective, nonexperimental clinical study was carried out correlating clinical features with a longitudinal analysis.

SPSS-20 software was used for estimation procedures.

Dichotomous variable "presence or absence of SD" has been studied and compared in both groups. Contrasts referred to equality of means of ANOVA like "duration of procedure," "age," and "duration of epiphora in years," Pearson's correlation coefficient like "age" and "duration of epiphora," or modeling explanatory ability of those predictor variables acting on probability of success, using the logistic regression model like "successful syringing at 3 and 6 months after operation," "sex," "bilaterality," "right/left side," "presence of granulomas," "presence of synechiae," "presence of postoperative granulomas," and "presence of SD," have been used.

3. Results

The study included 124 patients, in which 159 LP operations were performed, 102 LP (64.15%) did not have any anatomical variants, and 57 LP (35.84%) had anatomical variants. Out of these 57 LP with anatomical variants, 39 had SD (68.42%): in 21 of them it was mild (53.84%), in 15 it was moderate SD (38.46%), and in 3 it was severe SD (7.6%).

Other anatomical alterations were found in the remaining 18 LP (31.57%): one LP with concha bullosa (0%) and 17 LP with hypertrophic inferior turbinate (51%).

In the group with no anatomical alterations, 69 LP had a successful postoperative outcome (67.6%). In the SD group, 26 LP had a successful postoperative outcome (66.7%).

According to the LOGIT method, the difference between both groups in postoperative outcome was not significant ($p > 0.05$).

If we compare the success rate of the SD group (66.7%) and other anatomical alterations (44.1%) in our study, the difference is statistically significant ($p < 0.05$).

We also compared success rates in each SD group (mild, moderate, and severe).

The success rate for each group was as follows: 66.67% for mild SD; 66.60% for moderate SD; and 66.66% for severe SD.

In patients with severe SD (3), a previous septoplasty was necessary in order to make a correct TCL DCR possible later on, as a part of the same surgical procedure.

There were no complications during or after these procedures, no intraoperative bleeding, and no postoperative infection and all patients were discharged 4-5 hours after the operation.

4. Discussion

The demographic data collected for our sample agree with that found in worldwide medical literature, as far as sex [13–15], age [13–16], and race [15] are concerned.

There was a higher success rate in the SD group (66.7%) compared to the rest of anatomical alterations (success in this latter group amounted to 44.1%), but the former had a lower success rate when compared to the group with no anatomical alterations (67.6%).

The difference between those two groups was not statistically significant.

Most of septal deviations (69.6%) are inferior ridges or posterior deviations, so they did not need surgery. There is no need for previous or concomitant septoplasty in some cases like mild and moderate SD, because the success rate was similar.

All severe SD were operated on during the same procedure; therefore, access to the middle meatus in the nasal fossa presented no difficulties.

One month and three months later, most patients scored 0 to 1 on the Munk scale.

Stenosis, however, could recur due to fibrosis. It could change the scores from 0 to 2 over the course of the 6 months following surgery, so the success rate would decrease. After 6 months, the success rate remained stable.

We have not found articles referring to surgical success in patients with SD in scientific literature.

5. Conclusion

In our study, we found no significant statistical differences between the success rate in the two groups. Furthermore, we could avoid previous or concomitant septoplasty in some cases like mild and moderate SD because the success rate was similar to nonseptal deviation group. So we avoided risk of surgical complications like synechiae or granulomas.

Disclosure

The current address for Alberto Raposo is University Hospital Los Arcos del Mar Menor (UHAM), San Javier, 30739 Murcia, Spain.

Competing Interests

Alberto Raposo confirms that each co-author meets the requirements for authorship and has signed the Authorship Criteria Statement. No conflicting relationship exists.

References

[1] H. A. Weil, *Basic Dacryology. Diagnosis and Treatment*, 1987.

[2] J. A. Katowitz, D. A. Hollsten, and J. V. Linberg, *Lacrimal Surgery*, Churchill Livingstone, New York, NY, USA, 1988.

[3] J. C. Adan, *The Lacrimal System: Diagnosis, Management and Surgery*, 2006.

[4] G. A. Shun-Shin and G. Thurairajan, "External dacryocystorhinostomy—an end of an era?" *British Journal of Ophthalmology*, vol. 81, no. 9, pp. 716–717, 1997.

[5] H. Stammberger and W. Posawetz, "Functional endoscopic sinus surgery. Concept, indications and results of the Messerklinger technique," *European Archives of Oto-Rhino-Laryngology*, vol. 247, no. 2, pp. 63–76, 1990.

[6] F. Alañón, *Estudio comparativo de la obstrucción del sistema nasolagrimal mediante DCR endocanalicular con Láser Diodo y DCR externa [Ph.D. thesis]*, 2008.

[7] C. Suárez, L. M. Gil-Carcedo, J. Marco, J. Medina, P. Ortega, and J. Trinidad, *Tratado de Otorrinolaringología y Cirugía de Cabeza y Cuello*, Proyectos Médicos, Madrid, Spain, 2007.

[8] S. Jamie, "The incidence of concha bullosa and its relationship to nasal septal deviation and paranasal sinus disease," *American Journal of Neuroradiology*, vol. 25, no. 9, pp. 1613–1618, 2004.

[9] P. S. Hechl, R. C. Setliff, and M. Tschabitscher, *Endoscopic Anatomy of the Paranasal Sinuses*, Springer, New York, NY, USA, 1997.

[10] P. L. Munk, D. T. C. Lin, and D. C. Morris, "Epiphora: treatment by means of dacryocystoplasty with balloon dilation of the nasolacrimal drainage apparatus," *Radiology*, vol. 177, no. 3, pp. 687–690, 1990.

[11] B. Guyuron, C. D. Uzzo, and H. Scull, "A practical classification of septonasal deviation and an effective guide to septal surgery," *Plastic and Reconstructive Surgery*, vol. 104, no. 7, pp. 2202–2209, 1999.

[12] J. Hong-Ryul, L. Joo-Yun, and J. Woo-Jin, "New description method and classification system for septal deviation," *Journal of Rhinology*, vol. 14, no. 1, pp. 27–31, 2007.

[13] J. E. Hong, M. P. Hatton, M. L. Leib, and A. M. Fay, "Endocanalicular laser dacryocystorhinostomy: analysis of 118 consecutive surgeries," *Ophthalmology*, vol. 112, no. 9, pp. 1629–1633, 2005.

[14] P. Holzman, "Características clínicas y sociodemográficas de pacientes con epífora estudiada por dacriocistografía," *Archivos de Oftalmologia de Buenos Aires*, vol. 80, no. 4, 2009.

[15] J. Roussos and A. Bouzas, "Essai d'explication par des facteurs hormonaux de la grande fréquence d'apparition de la dacryocystite chronique chez les femmes plutôt que chez les hommes," *Société Française d'Ophtalmologie*, vol. 86, pp. 96–99, 1973.

[16] U. Schaudig and H. Meyer-Rüsenberg, "Epiphora. Age-related changes of the ocular surface, eyelid function and the efferent tear ducts," *Ophthalmologe*, vol. 106, no. 3, pp. 229–234, 2009.

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Clinical Study

Comparison of Transcanalicular Multidiode Laser Dacryocystorhinostomy with and without Silicon Tube Intubation

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Aim. To compare the surgical outcomes of surgery with and without bicanalicular silicon tube intubation for the treatment of patients who have primary uncomplicated nasolacrimal duct obstruction. *Methods*. This retrospective study is comprised of 113 patients with uncomplicated primary nasolacrimal duct obstruction. There were 2 groups in the study: Group 1 ($n = 58$) patients underwent transcanalicular diode laser dacryocystorhinostomy surgery with bicanalicular silicon tube intubation and Group 2 ($n = 55$) patients underwent transcanalicular diode laser dacryocystorhinostomy surgery without bicanalicular silicon tube intubation. The follow-up period was 18.42 ± 2.8 months for Group 1 and 18.8 ± 2.1 months for Group 2. *Results*. Success was defined by irrigation of the lacrimal system without regurgitation and by the absence of epiphora. Success rates were 84.4% for Group 1 and 63.6% for Group 2 ($P = 0.011$). Statistically a significant difference was found between the two groups. *Conclusion*. The results of the study showed that transcanalicular diode laser dacryocystorhinostomy surgery with bicanalicular silicon tube intubation was more successful than the other method of surgery. Consequently, the application of silicone tube intubation in transcanalicular diode laser dacryocystorhinostomy surgery is recommended.

1. Introduction

Nasolacrimal duct obstruction (NLDO) is the most common cause of chronic dacryocystitis and in this case the only treatment option is surgery [1, 2]. Although the external surgical approach still is the gold standard with the highest success rate, the most recent stage in the development of dacryocystorhinostomy (DCR) is the endocanalicular or transcanalicular approach. In this approach, a probe with a red light on the end is inserted outside punctum and is moved toward the nasal wall [3]. Subsequently, nasal osteotomy is performed by diode laser energy [4].

Laser-assisted DCR application began with Massaro et al. in 1990; and, in addition to argon laser diode, potassium titanyl phosphate (KTP), holmium YAG, CO_2, Nd:YAG, and erbium lasers have also been used until today [5–7]. New ostium is created by these lasers from an intranasal or transcanalicular approach.

Flexible endoscopes (0.3–0.7 mm diameter) modified from gastroduodenal endoscopes were developed for transcanalicular surgery [8]. By extending the diameter of the endoscopes and increasing the pixels of imaging, better quality results have been obtained.

Some authors have suggested the use of silicon tube intubation in NLDO surgery [9, 10], while some prefer using silicon tubes only for definitive indications (canalicular damage, lacrimal sac inflammation, secondary surgery, small and contracted sacs, etc.) [11, 12].

The purpose of this retrospective study was to compare the surgical outcomes of transcanalicular diode laser dacryocystorhinostomy (TDL-DCR) surgery with and without bicanalicular silicon tube intubation in the treatment of a series of 113 patients with primary uncomplicated nasolacrimal duct obstruction.

2. Subjects and Methods

2.1. Subjects. A series of 113 patients who had not previously undergone this surgery were operated on for NLDO between 2010 and 2013. The study was carried out in accordance with the tenets of the Declaration of Helsinki. Approval for the study was granted by the Clinical Research Ethics Committee of GATA Haydarpasa Training Hospital (1491-59-14/1539) and informed consent was obtained from all the patients. A retrospective review was made of the 2 groups of patients. Group 1 is comprised of 58 patients who underwent TDL-DCR surgery with bicanalicular silicon tube intubation, and Group 2 is comprised of 55 patients who underwent TDL-DCR surgery without bicanalicular silicon tube intubation.

After complete ophthalmic examination, nasolacrimal duct obstruction was confirmed with lacrimal irrigation and dacryocystography with Lipiodol® preoperatively in each case. Blood tests were obtained from all patients to analyze systemic diseases.

The patients were selected according to the following criteria: (i) no history of nasolacrimal duct surgery; (ii) no canalicular obstruction; (iii) no history of traumatic injury to the ocular or nasal region; (iv) no concomitant nasal pathology, such as septum deviation, concha bullosa, nasal polyposis, and atrophic rhinitis; (v) absence of active infective dacryocystitis; (vi) absence of dry eye and lower lid laxity.

2.2. Methods. All operations were performed as in another previous study [13]. All operations were performed under local anesthesia. Before the surgery, topical anesthetic drops (oxybuprocaine hydrochloride 0.4%) were put on the conjunctiva and cornea. Then intranasal, infraorbital, and lateral nasal side anesthesia were applied with a solution mixture of epinephrine hydrochloride and lidocaine.

After dilating the lacrimal puncta, the fiber was inserted through the canaliculus to the wall of the sac. The feeling of a hard stop is essential during the insertion process. With the endoscopic visualization of the nasal cavity, the red light reflex of the fiber is clearly seen on the nasal wall of the middle turbinate plane (Figure 1). In this way, the target tissue was determined by the laser light guide.

Diode laser (INTERmedic™ diode S30 OFT 980 nm) parameters were settled at 10 W in 500 ms pulse mode potency, taking care not to prolong each impact to avoid overheating the structures. After reaching the nasal cavity, the osteotomy was expanded sufficiently with the fiber manipulation. A Crawford-type aspirator was used to displace the middle turbinate medially to protect the septum and middle turbinate and to maintain adequate exposure to the surgical site. Laser application was continued until the width of the new ostium becomes greater than 5 mm diameter (Figure 2). The size of osteotomy was controlled by the use of the nasal endoscope. The laser shots were between 28 and 45 shots at 10 watts. At the end of surgery, the laser probe was removed and lacrimal irrigation with saline solution was administered.

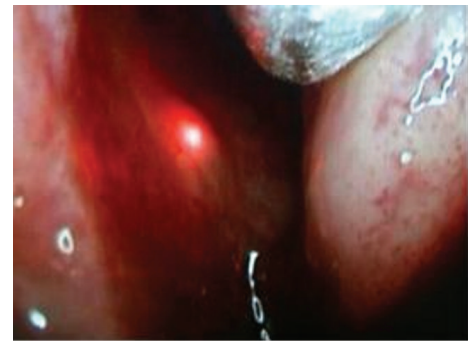

Figure 1: The red light reflex of the fiber is clearly seen on the nasal wall of the middle turbinate.

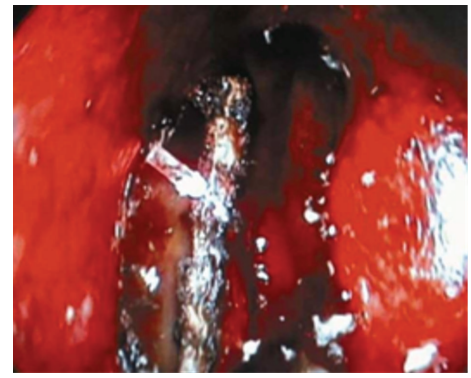

Figure 2: The ostium was expanded sufficiently with the fiber manipulation.

In addition to the surgery in Group 1 ($n = 58$), bicanalicular silicone intubation was performed. Silicone extensions of the tube were tied to each other and then were left free in the nasal cavity (Figure 3). Tamponade was applied to the nasal cavity to ensure control of the bleeding.

Postoperatively, antibiotic and steroid eye drops, nasal steroid spray, and also nasal saline were to be used four times a day for 2 weeks. Additionally, oral antibiotic was to be used for 7 days.

Follow-up postoperative examinations were carried out on the first day, in the first week, in the first month, in the 3rd month, and then at 3-month intervals. Silicone tube was removed 3 months after intubation. In follow-up visits, the patency of the lacrimal drainage system was checked. Resolution of symptomatic epiphora and lack of resistance in nasolacrimal saline irrigation were defined as success. The follow-up time was at least 12 months.

2.3. Statistical Analysis. Data analyses were performed using SPSS 14.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). The normal distribution of the considered variables was first evaluated using the Shapiro-Wilk test. The data was presented as the mean ± standard deviation for the

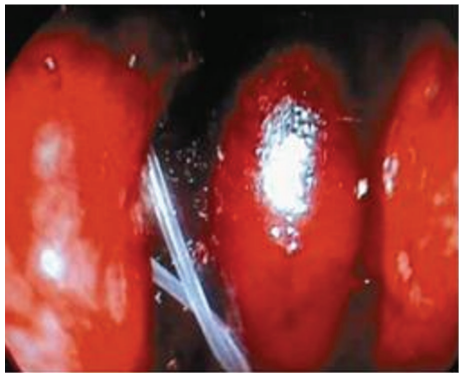

Figure 3: The silicon tubes were left free in the nasal cavity.

continuous variables, and the number of cases was used for the categorical ones. Independent samples t-test was used to compare the means between Group 1 and Group 2. The differences between the groups were analyzed by Chi-square tests. A value of $P \leq 0.05$ was accepted as statistically significant.

3. Results

The study is comprised of 113 patients: Group 1 was composed of 58 patients (28 males, 30 females) with a mean age of 33.6 ± 11.57 (21–65) years and Group 2 was composed of 55 patients (21 males, 34 females) with a mean age of 37.4 ± 10.01 (21–65) years. Final success rates were (49/58) 84.4% for Group 1 and (35/55) 63.6% for Group 2 ($P = 0.011$). The mean surgical time for Groups 1 and 2 was 15.96 ± 3.01 and 13.74 ± 3.66 mins (range: 9–21 mins in both groups), respectively. The mean surgical time was longer due to silicon tube tying in Group 1 and there was a statistically significant difference among the groups ($P = 0.001$). The mean total laser energy of Groups 1 and 2 was 670.52±49.18 and 651.09±49.57 Joules (range: from 420 to 720 Joules in both groups), respectively. There was no statistically significant difference among the groups in terms of total laser energy ($P = 0.951$).

In Group 1, endoscopic examinations showed granulomas in 3 patients. These granulomas were removed by endoscopic procedures. In 6 cases, the result was evaluated as a failure, as there was mucosal scarring around the osteotomized area, and reobstruction occurred between 3 and 6 months postoperatively in 4 patients and between 6 and 12 months in 2 patients. In the 2nd month, 1 patient in Group 1 developed an episode of infection, which was immediately treated with medical therapy. Except for that patient, there were no other complications such as erosion of the punctum, fistulation to skin, and removal of the tubes. In Group 2, endoscopic examinations showed scarring of the internal ostium requiring secondary surgery in 20 of the patients. Reobstruction occurred between 1 and 3 months in 12 patients, between 3 and 6 months in 6 patients, and between 6 and 12 months in 2 patients postoperatively. The follow-up period was 18.4±2.8 months for Group 1 and 18.8 ± 2.1 months for Group 2.

4. Discussion

Transcanalicular diode laser dacryocystorhinostomy (TDL-DCR) is a minimally invasive surgical procedure, which has the great advantage of accessing the operating field through anatomic pathways. It minimizes trauma to surrounding tissue, avoids unnecessary surgical skin scars, and provides precise cutting and removal of tissue by ablation. In addition, TDL-DCR causes minimum pain and minimum nasal bleeding. It is also easier and faster to perform compared to the classical dacryocystorhinostomy. Silicon tube intubation with DCR surgery is used to prevent the blocking of the lacrimal passage and to provide epithelization. Since silicon is an inert substance, it does not damage the conjunctiva and can be well-tolerated in the canaliculi.

As mentioned above, the use of silicon tube intubation has been suggested for patients with coexisting canalicular diseases, contracted or scarred lacrimal sacs, and persistent congenital nasolacrimal duct obstructions. Allen et al. [14] evaluated 242 cases retrospectively and showed no statistically significant difference between failure and age but a statistically significant difference between failure and silicon tube intubation. In their study, it was reported that formation of granulomatous tissue at the site of osteotomy is one of the most important failure factors in surgery with silicon tube intubation.

In literature, there are few studies about DCR surgery with and without silicon tubes. While some studies have reported no statistically significant advantage of using DCR with silicon stents over the DCR without stents [15–17], in the other studies, intubation is recommended in DCR surgery [9].

Feng et al. [16] concluded that no benefit was found in silicon tube intubation in primary DCR based on a meta-analysis of primary dacryocystorhinostomy with and without silicon intubation that included 9 trials involving 514 cases.

In the current study, the TDL-DCR surgery group with silicon tubes had a success rate of 84.4% (49/58), while the other group without tubes had a success rate of 63.6% (35/55), with a significant difference between these groups ($P < 0.05$). The success rates of both groups in the current study were similar to previous reports. Success rates have been reported to vary between 80% and 99% in external DCR surgery and between 58% and 97% in endoscopic nasal procedures [18–22]. The success of this combination with silicon tubes in TDL-DCR, possibly occurring ostium closure, is due to inhibition by the silicon tube during wound healing.

There were a total of 29 failures in this study: 9 in Group 1 and 20 in Group 2. Formation of granulomatous tissue occurred in 3 failed cases in Group 1. In this group, dacryocystitis was also observed in 1 patient. In addition, endoscopic examinations showed scarring of the internal ostium in 6 patients in Group 1 and in 20 patients in Group 2.

Rebeiz et al. [23] suggested 4 to 6 weeks for the duration of silicon tube intubation. To prevent the formation of granuloma, Kong et al. [24] suggested not removing the tubes before 8 weeks. Häusler and Caversaccio [25] reported that the tubes were well-tolerated by the patients and permit drainage of the nasolacrimal ducts for months and even years.

In that study, the tubes remained in place for 9 months on average. In the current study, the silicon tubes were removed 3 months after surgery. The bicanalicular tubes in the lacrimal ducts were well-tolerated by all patients without notable problems except in 1 patient who developed an infection. The great number of female participants compared to the males was consistent with previous findings [18, 20].

This study concluded that the success rate was different in the two TDL-DCR surgery groups with and without silicon tubes. Silicon tube intubation was advantageous for patients who were undergoing their first dacryocystorhinostomy surgery for nasolacrimal duct obstruction. On the basis of these different outcomes, bicanalicular silicon tube intubation should be used in TDL-DCR surgery for patients with primary nasolacrimal duct obstruction.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Taner Kar wrote the statistical analysis plan, cleaned and analyzed the data, and drafted and revised the paper. Enver Cesmeci and Tuncay Topal analyzed the data and drafted and revised the paper.

Acknowledgments

The authors thank Yildiray Yildirim for initiating the project, designing the data collection tools, and monitoring data collection for the whole trial. He is the guarantor of the study.

References

[1] J. Hartikainen, J. Antila, M. Varpula, P. Puukka, H. Seppä, and R. Grénman, "Prospective randomized comparison of endonasal endoscopic dacryocystorhinostomy and external dacryocystorhinostomy," *Laryngoscope*, vol. 108, no. 12, pp. 1861–1866, 1998.

[2] S. Lee and M. T. Yen, "Laser-assisted dacryocystorhinostomy: a viable treatment option?" *Current Opinion in Ophthalmology*, vol. 22, no. 5, pp. 413–418, 2011.

[3] B. Parente Hernández, A. Sentieri Omarrementería, and J. Junceda Moreno, "Corrective techniques of lacrimal obstruction in the vertical system," *Archivos de la Sociedad Espanola de Oftalmologia*, vol. 87, no. 5, pp. 139–144, 2012.

[4] E. B. Strong, "Endoscopic dacryocystorhinostomy," *Craniomaxillofacial Trauma & Reconstruction*, vol. 6, no. 2, pp. 67–74, 2013.

[5] B. M. Massaro, R. S. Gonnering, and G. J. Harris, "Endonasal laser dacryocystorhinostomy. a new approach to nasolacrimal duct obstruction," *Archives of Ophthalmology*, vol. 108, no. 8, pp. 1172–1176, 1990.

[6] R. S. Gonnering, D. B. Lyon, and J. C. Fisher, "Endoscopic laser-assisted lacrimal surgery," *American Journal of Ophthalmology*, vol. 111, no. 2, pp. 152–157, 1991.

[7] J. J. Woog, R. Metson, and C. A. Puliafito, "Holmium: YAG endonasal laser dacryocystorhinostomy," *American Journal of Ophthalmology*, vol. 116, no. 1, pp. 1–10, 1993.

[8] K. H. Emmerich, M. Luchtenberg, H. W. Meyer-Ruesenberg, and J. Steinhauer, "Dacryoendoscopy and laser dacryoplasty: technique and results," *Klinische Monatsblätter für Augenheilkunde*, vol. 211, no. 6, pp. 375–379, 1997.

[9] S. Zuhair Al-Asadi and A. Muhammed Al-Abbasi, "Efficacy of diode laser transcanalicular dacryocystorhinostomy in acquired nasolacrimal duct obstruction," *Gomal Journal of Medical Sciences*, vol. 9, no. 2, pp. 152–154, 2011.

[10] J. J. Woog, R. H. Kennedy, P. L. Custer, S. A. Kaltreider, D. R. Meyer, and J. G. Camara, "Endonasal dacryocystorhinostomy: a report by the American Academy of ophthalmology," *Ophthalmology*, vol. 108, no. 12, pp. 2369–2377, 2001.

[11] T. W. Doucet and J. J. Hurwitz, "Canaliculodacryocystorhinostomy in the treatment of canalicular obstruction," *Archives of Ophthalmology*, vol. 100, no. 2, pp. 306–309, 1982.

[12] Ş. Ari, A. K. Cingü, A. Şahin, R. Gün, V. Kiniş, and I. Çaça, "Outcomes of revision external dacryocystorhinostomy and nasal intubation by bicanalicular silicone tubing under endonasal endoscopic guidance," *International Journal of Ophthalmology*, vol. 5, no. 2, pp. 238–241, 2012.

[13] Y. Yildirim, M. Salihoglu, T. Kar et al., "Postoperative changes in olfactory function after transcanalicular diode laser dacryocystorhinostomy," *Ophthalmic Plastic and Reconstructive Surgery*, vol. 31, no. 2, pp. 94–97, 2015.

[14] K. Allen and A. J. Berlin, "Dacryocystorhinostomy failure: association with nasolacrimal silicone intubation," *Ophthalmic Surgery*, vol. 20, no. 7, pp. 486–489, 1989.

[15] A. S. Al-Qahtani, "Primary endoscopic dacryocystorhinostomy with or without silicone tubing: a prospective randomized study," *American Journal of Rhinology and Allergy*, vol. 26, no. 4, pp. 332–334, 2012.

[16] Y.-F. Feng, J.-Q. Cai, J.-Y. Zhang, and X.-H. Han, "A meta-analysis of primary dacryocystorhinostomy with and without silicone intubation," *Canadian Journal of Ophthalmology*, vol. 46, no. 6, pp. 521–527, 2011.

[17] G. Smirnov, H. Tuomilehto, M. Teräsvirta, J. Nuutinen, and J. Seppä, "Silicone tubing is not necessary after primary endoscopic dacryocystorhinostomy: a prospective randomized study," *American Journal of Rhinology*, vol. 22, no. 2, pp. 214–217, 2008.

[18] R. D. Henson, H. L. Cruz, R. G. Henson, M. J. Ali, and H. Kakizaki, "Postoperative application of mitomycin-C in endocanalicular laser dacryocystorhinostomy," *Ophthalmic Plastic and Reconstructive Surgery*, vol. 28, no. 3, pp. 192–195, 2012.

[19] D. L. McLachlan, G. M. Shannon, and J. C. Flanagan, "Results of dacryocystorhinostomy: analysis of the reoperations," *Ophthalmic Surgery*, vol. 11, no. 7, pp. 427–430, 1980.

[20] V. K. Pal, A. Agrawal, S. Suman, and V. B. Pratap, "Transcanalicular endoscope combined laser-assisted dacryocystorhinostomy," *Oman Journal of Ophthalmology*, vol. 6, no. 2, pp. 99–102, 2013.

[21] D. Horix and H. G. Struck, "Long term patency rate of the external dacryocystorhinostomy. A retrospective study in the years 1991–2000 at the University Eye Hospital in Halle," *Ophthalmologe*, vol. 101, no. 3, pp. 268–277, 2004.

[22] H. Erdöl, N. Akyol, H. I. Imamoglu, and E. Sözen, "Long-term follow-up of external dacryocystorhinostomy and the factors affecting its success," *Orbit*, vol. 24, no. 2, pp. 99–102, 2005.

[23] E. E. Rebeiz, S. M. Shapshay, J. H. Bowlds, and M. M. Pankratov, "Anatomic guidelines for dacryocystorhinostomy," *Laryngoscope*, vol. 102, no. 10, pp. 1181–1184, 1992.

[24] Y. T. Kong, T. I. Kim, and B. W. Kong, "A report of 131 cases of endoscopic laser lacrimal surgery," *Ophthalmology*, vol. 101, no. 11, pp. 1793–1800, 1994.

[25] R. Häusler and M. Caversaccio, "Microsurgical endonasal dacryocystorhinostomy with long term insertion of bicanalicular silicone tubes," *Archives of Otolaryngology—Head and Neck Surgery*, vol. 124, no. 2, pp. 188–191, 1998.

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Research Article

Evaluating the Functionality of Conjunctiva Using a Rabbit Dry Eye Model

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Purpose. To assess the conjunctival functionality in a rabbit dry eye (DE) model. *Methods.* Nictitating membrane, lacrimal and Harderian glands were surgically excised from male New Zealand white rabbits using minimally invasive surgery. Fluorescein/rose Bengal staining of ocular surface (OS) and Schirmer test were done before (BE) and after excision (AE). The expression of interleukin- (IL-) 1β, tumor necrosis factor- (TNF-) α, and MUC5AC proteins were estimated by immunoblotting from conjunctival impression cytology specimens. MUC5AC mRNA was quantified as well. The effect of epithelial sodium channel (ENaC) blockers on tear production and potential differences (PD) of OS were assessed under anesthesia in rabbits with and without surgery. *Results.* Increase in corneal and conjunctival staining was observed 1 month AE compared to BE. Schirmer tests failed to show decrease in tear production. Elevated IL-1β, and TNF-α, 1 month AE indicated inflammation. MUC5AC expression was elevated 1 month AE. ENaC blockers did not improve tear production in rabbit eyes AE but characteristic changes in PD were observed in rabbits with surgery. *Conclusions.* DE biomarkers are important tools for OS assessment and MUC5AC expression is elevated in rabbit DE. PD measurement revealed significant electrophysiological changes in rabbits with surgery.

1. Introduction

Tear film (TF) constantly protects the exposed surface of the eye, the cornea, and the conjunctiva from environmental stresses including desiccation, temperature change, physical injury, and infections [1]. By providing optimal concentrations of electrolytes, proteins, mucin, and lipids, the TF is critical in the maintenance of corneal transparency and good vision [1]. Dry eye disease (DED) is a multifactorial dysfunction of the TF, resulting in symptoms of discomfort, visual disturbance, and even loss of vision due to damage to the ocular surface [2]. DED is generally acknowledged to be, in large part, due to reduced secretion or increased evaporation of the tear fluid, resulting in subsequent increase in osmolarity and inflammation at the ocular surface [2]. Since DED represents a diverse group of conditions that manifest as inadequate ocular surface lubrication, restoration of a sufficient tear volume remains the mainstay of current dry eye (DE) treatment.

Although lacrimal gland (LG) is considered the main source of tears [3], increasing evidence suggests that under certain conditions conjunctival epithelium has the capacity to be the primary source of TF [1]. Removal of the main LG of squirrel monkeys does not lead to keratoconjunctivitis sicca (KCS) [4]. In humans, up to 86% of patients with epiphora who underwent palpebral dacryoadenectomy (PDA) did not develop DE, and in up to 50% of such patients the epiphora persisted [5, 6]. Although accessory LGs were believed to be mostly responsible in these cases, the conjunctiva certainly plays a role as a compensatory tissue. The human conjunctiva occupying 17 times more surface area than the cornea has the potential to be the primary modulator of tear volume and component [7].

We are interested in understanding the physiology of conjunctival epithelium so as to maximize its fluid secretion capacity as an alternative to DED treatment. A rabbit model with intact conjunctiva and equal DE phenotype bilaterally is ideal in such research. We created a DE model in rabbits by surgical excision of the nictitating membrane (NM), Harderian gland (HG), and main LG [8]. Surprisingly, the tear secretion was not significantly reduced by these operations. Although DE associated ocular surface phenotype and inflammatory biomarkers elevated in the immediate postoperative period, they gradually decreased over 4-month duration to near preoperative level without therapeutic intervention [8]. These findings suggest that the rabbit ocular surface can potentially compensate for the loss of these seemingly vital ocular surface structures, including the main LG. The results also indicate that, in acute DE condition (as created in our experiment), ocular surface injury and inflammation can be mostly reverted. To gain further insight into the exact mechanisms of conjunctiva mediated tear compensation, the present study further explored methods of conjunctival characterization in this mixed mechanism rabbit DE model.

2. Methods

2.1. Experimental Animals and Ethics Statement. Male New Zealand white rabbits ($N = 8$, 16 eyes, Harlan Sprague Dawley, Indianapolis, IN, USA) weighing 2.0–2.5 kg were used for this study. The rabbits were reared under standard laboratory conditions (22 ± 2°C, 40% ± 5% relative humidity, and a 12-hour light-dark cycle) with free access to food and water throughout the experiment. The study was conducted in compliance with the Tenets of the Declaration of Helsinki and ARVO statement for the use of animals in ophthalmic and visual research. The protocol was approved by the University of Arizona (Tucson, AZ, USA) Institutional Animal Care and Use Committee (protocol# 14-511). All surgeries were performed by skilled surgeons (YN and MW).

2.2. Operative Procedure. The surgical protocol for resection of main LG, HG, and NM was published previously [8] which was modified from established procedures [9, 10]. Identical procedure was performed on the left and right eye.

2.3. Evaluations. The rabbits were assessed before excision (BE) and after excision (AE). To minimize slit lamp finding artifact from other tests, the evaluations were carried out in two days in the following sequence of each eye. The first day begins with corneal fluorescein test, followed immediately by rose Bengal staining and CIC. On the second day, Schirmer tests, without (Schirmer I test, SIt) and with anesthesia (Schirmer II test, SIIt), were performed separately in the morning and afternoon.

2.4. Corneal Fluorescein and Rose Bengal Staining Tests. The eyes of all rabbits were examined under a slit lamp microscope (GR-54, Gilras LLC, Miami, FL) by the same ophthalmologist (YN) following protocol described previously [8].

2.5. Schirmer I and II Tests. Both SIt and SIIt were carried out in our study. The SIt was performed as per the protocol described previously [8]. For SIIt, one drop of 0.5% proparacaine hydrochloride (Bausch and Lomb, Tampa, FL, USA) was placed and the excess fluid was blotted away with soft paper tissue, prior to the insertion of the filter paper strips (Alcon Laboratories, Inc., Fort Worth, TX, USA) in the lower lateral one-third of conjunctival fornix and eyelids closed by gentle force for 5 mins. Both tests were performed three times and the average score was used for analysis.

2.6. Conjunctival Impression Cytology. Conjunctival impression cytology (CIC) was performed as per the protocol published [8]. The filter paper discs were peeled off and immediately placed in either 500 μL Trizol solution (Invitrogen, CA, USA) for RNA isolation or 100 μL of radio immunoprecipitation assay (RIPA) buffer (Teknova, CA, USA) for protein isolation.

2.7. RNA Isolation and cDNA Synthesis. Total RNA was isolated from the CIC specimens in Trizol solution according to manufacturer's instructions (Invitrogen, CA, USA). RNA concentrations were estimated by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington DE, USA) in 1 μL volume. Purity of the RNA was assessed by the ratio of absorbance at 260/280 nm. A ratio of 1.9 to 2 was considered to be good quality RNA specimen and used for further experiments. The first strand of cDNA was synthesized with QuantiTect® Reverse Transcription Kit (Qiagen, Valencia, CA, USA) using 500 ng total RNA according to the manufacturer's instructions.

2.8. Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-qPCR). The RT-qPCR reactions were set using SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. The primer sequences for MUC5AC were as follows: MUC5AC-F: CCCCAACGTCAAGAACAACT and MUC5AC-R: TCAAACAGGCAGTTCGAGTG [11]. The RT-qPCR was performed on StepOnePlus™ Real-Time PCR System (Applied Biosystems) with the following cycling conditions: 15 min 95°C, 40 cycles of 15 sec 95°C, and 30 sec 60°C. The fluorescence was recorded during elongation step in each cycle. A melting curve analysis was performed at the end of each PCR by gradually increasing the temperature from 60 to 95°C while recording the fluorescence. A single peak at the melting temperature of the PCR product confirmed primer specificity. To compare between different runs, a fixed fluorescence threshold for derivation of C_T value for all runs was used. Three technical replicates were performed to evaluate the relative quantification.

2.9. Relative Quantification of mRNA Level. Relative quantification of MUC5AC expression in rabbit CIC specimens was performed BE and 1 month AE. The fold change in MUC5AC expression was relative to the internal housekeeping gene, β-actin (endogenous control). Mean fold change in MUC5AC expression was calculated using $2^{-\Delta\Delta C_T}$ method, where

$\Delta\Delta C_T = (C_{T\,Gene} - C_{T\,Actin})_{After\ Excision} - (C_{T\,Gene} - C_{T\,Actin})_{Before\ Excision}$. Difference between C_T for MUC5AC and β-actin mRNA in each specimen was used to calculate level of target mRNA relative to that of β-actin mRNA in the same specimen [8].

2.10. Immunoblotting. Total cell lysate proteins were isolated from CIC in radio immunoprecipitation assay (RIPA) buffer with 1x HALT protease and phosphatase inhibitor single use inhibitor cocktail (Thermoscientific, Rockford, IL, USA) by incubating on ice for 30 min. Protein concentration was determined by Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, NY). Specimens were mixed with Laemmli sample buffer (Bio-Rad laboratories, Inc. Hercules, CA, USA) containing β-mercaptoethanol and heated at 95°C for 10 min. Specimens were then immunoblotted and analyzed as per the protocol published previously [8]. The primary rabbit monoclonal antibodies to IL-1β, TNF-α, and MUC5AC (Abcam, Cambridge, MA, USA) were used at a dilution of 1: 200.

2.11. Effect of Epithelial Sodium Channel Blockers on Conjunctival Tear Secretion. To test the effects of epithelial sodium channel (ENaC) blockers on tear secretion, amiloride and benzamil were administered topically to the right eyes ($n = 8$) of the operated rabbits 2 months AE. A 0.1% of amiloride and benzamil [12] (both from Sigma-Aldrich, Inc. St. Louis, MO, USA) were prepared in sterile buffered saline solution (BSS) and tested in separate experiments. The right eyes were allocated to ENaC blockers and left eyes to BSS as control. A 50 μL of ENaC blocker eye drops or BSS was instilled into the lower conjunctival sac by a micropipette at the beginning of the experiments. SIIt was performed before and at 5 min, 15 min, 30 min, 60, and 90 min after application of amiloride or benzamil.

2.12. Open-Circuit Potential Difference Measurements at the Rabbit Ocular Surface. Potential difference (PD) is generated by electrogenic Cl^- secretion and Na^+ reabsorption across superficial cell apical membrane of the corneal and conjunctival epithelia [13]. PD measurement is a sensitive modality in detecting transepithelial electrolyte conductance at the ocular surface [14]. Therefore, to help delineate the underlying physiological change which contributes to the increased output of tears by the rabbit conjunctiva AE, open-circuit PD was measured with a method modified from a previously established protocol in mice [14]. Briefly, the rabbits were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine (Sigma-Aldrich, MO, USA) and placed on a heating pad in a stereotaxic device with conjunctival and corneal tissues exposed and faced upwards. Two different solutions were perfused in series over the ocular surface at a rate of 10 mL/min using a pinch valve perfusion system (PS-8H; Bioscience Tools, San Diego, CA, USA) and peristaltic pump (13-876-1; Fisher Scientific, Pittsburgh, PA, USA) with $1/16''$ inner-diameter plastic tubing.

First, phosphate-buffered saline (1x PBS) was perfused for 5 min to establish a stable baseline, and then 100 μM amiloride (Sigma-Aldrich) prepared in 1x PBS was perfused. A low powered wall vacuum attached to $1/16''$ ID tubing was placed next to the fluid bolus covering the ocular surface to keep the volume constant and avoid fluid runoff. The PDs were measured with a high-impedance digital voltmeter, IsoMilivolt Meter (World Precision Instruments, Sarasota, FL, USA) with two Ag/AgCl electrodes connected through a 1 M KCl agar bridge. One probe was placed in contact with the ocular fluid, and the other was placed subcutaneously in the rabbit's mid-back. The PDs were measured on operated rabbit eyes ($n = 4$) 5 months AE and compared with normal rabbit eyes ($n = 4$) as controls.

2.13. Data Analysis and Statistics. Data in figures are presented as mean Standard Error Method, the bars representing standard errors. Statistical significance between two groups (BE and AE) was evaluated using unpaired 2-tailed t-test. A probability of P equal to 0.05 was considered significant (where applicable, $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$). The Spearman correlation analysis was employed to determine the correlation between every pair of the tests performed, BE and AE.

3. Results

3.1. Modification of the Operative Procedure. Chen et al. extracted the HG through an inferior orbital rim incision [9]. We found that this approach requires a long incision toward the medial canthus. In addition, massive hemorrhage tends to occur while excising the HG from between the medial rectus muscle and the anterior orbital wall. Gelatin sponge was used to achieve hemostasis during their surgeries. In our study, excision of the HG through the NM excision wound was much less invasive. Less hemorrhage and improved visibility of the surgical field ensured complete ablation of the HG (Figure 1). Li et al. extracted HG using similar method [10]. However, a 5 mm palpebral conjunctival incision was made in their study to extract lobes of the main LG. In our experience, such a small incision would not permit adequate access to all lobes of the LG, especially the intraorbital lobe, which is deeply embedded beneath the lateral orbital rim and separated by a dense membranous connective tissue from the superficial temporal lobe. No additional conjunctival incision was necessary in our procedure and hence the entire conjunctival surface is preserved. The skin incision only needed to cover the lateral two-thirds of the orbital rim in order to have a good exposure to adequately remove the infraorbital, temporal, and intraorbital lobes of the LG. A rabbit model with intact conjunctiva and equal DE phenotype bilaterally is ideal for our research to comparatively assess modalities that potentially stimulate conjunctival fluid secretions.

3.2. Ocular Surface Changes. As compared to BE, both fluorescein and rose Bengal staining increased on the cornea and conjunctiva (Figure 2) 1 month AE. Significantly higher staining scores ($P < 0.0001$ in both) demonstrated the presence of DE phenotype at the ocular surface. For all tests conducted, there were no significant differences found as a function of left versus right eye.

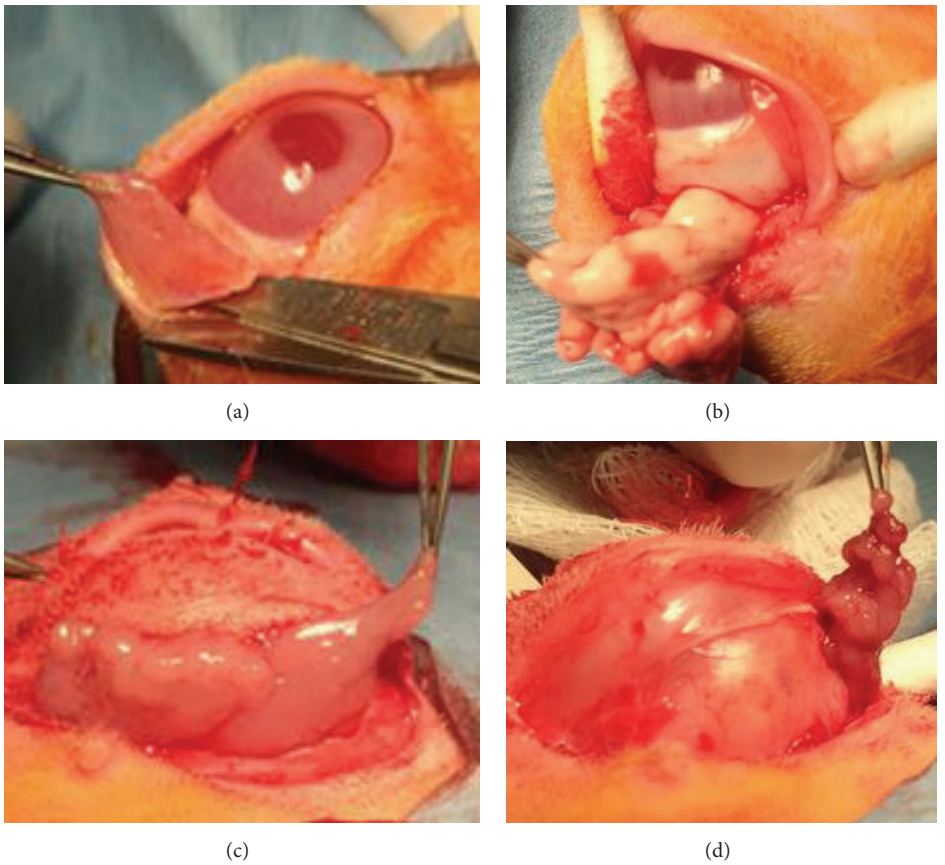

Figure 1: Major surgical steps involved in creating our rabbit dry eye model. (a) Nictitating membrane (NM) was removed at the base; (b) Harderian gland was separated and ablated through same wound as excision of NM (this was done to reduce hemorrhage); (c) removal of infraorbital and temporal lobes of the lacrimal gland; (d) removal of the deeply embedded intraorbital lobe of the lacrimal gland.

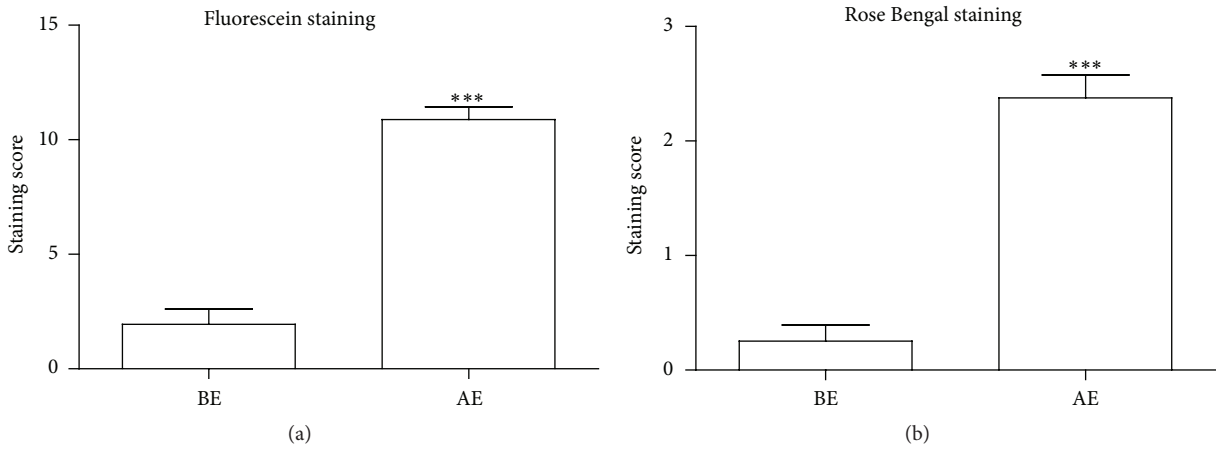

Figure 2: Comparison of fluorescein and rose Bengal staining of rabbit eyes before and 1 month after surgery. There were significant differences in fluorescein staining (a) and rose Bengal staining (b) ($^{***}P < 0.0001$) before excision (BE) and after excision (AE). Data are presented as mean Standard Error Method (SEM).

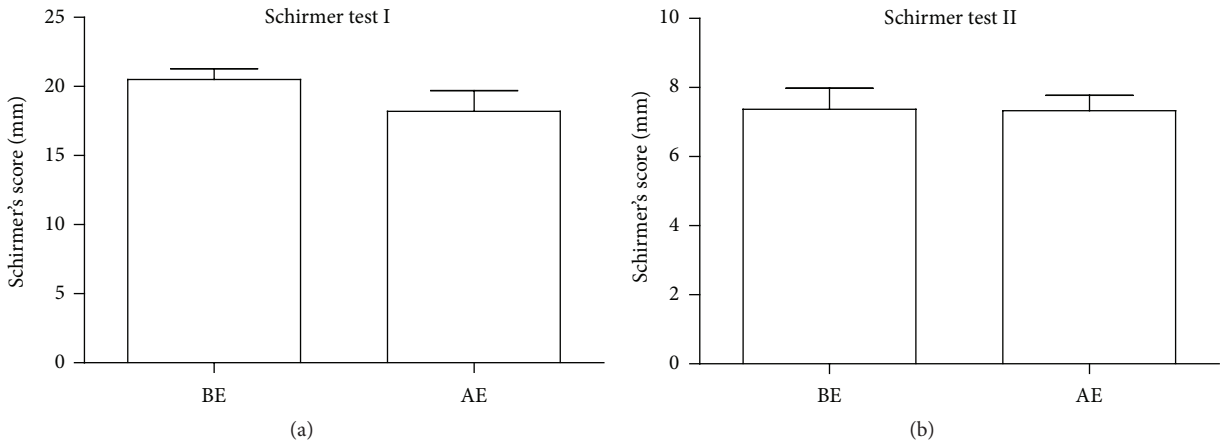

Figure 3: Comparison of Schirmer tests (I and II) before (BE) and 1 month after excision (AE). There were no significant differences in Schirmer scores BE and 1 month AE, either without anesthesia (Schirmer I) or with anesthesia (Schirmer II). Data are presented as mean Standard Error Method (SEM).

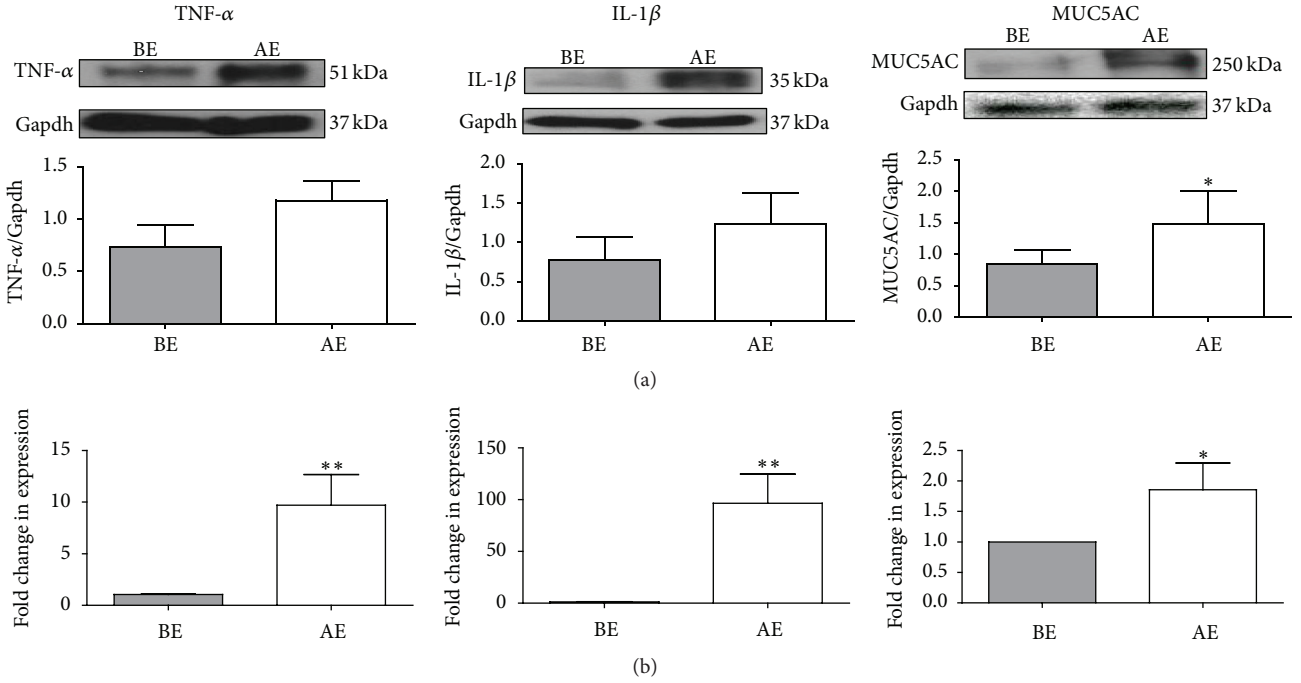

Figure 4: Quantification of mRNA and protein levels of inflammatory cytokines and MUC5AC in rabbit conjunctival impression cytology specimens BE and 1 month AE. For the proteins, the signal for the gene was normalized with the Gapdh signal from the same gene (a). For mRNA, the fold change in expression of genes is relative to endogenous control, β-actin (b). The data for mRNA of IL-1β and TNF-α (b) were referred from the previous publication [8]. The upregulation of mRNA correlated with the increase at protein level for the inflammatory cytokines and MUC5AC. Data are presented as mean Standard Error Method (SEM). For all graphs, bars show standard error (SE); statistical differences are shown ($^{*}P < 0.05$, $^{**}P < 0.01$).

3.3. Schirmer's Tests. In our study, large variations were noted in both Schirmer tests among eyes tested either BE or AE. With both SIt ($P = 0.104$) and SIIt ($P = 0.478$), no significant reduction in tear production was seen 1 month AE (Figure 3). There was, however, significant difference between the SIt and SIIt ($P < 0.0001$) either BE or AE, with tear secretion being lower under topical anesthesia.

3.4. Upregulation of Dry Eye Biomarkers. The protein levels of DED associated inflammatory cytokines (TNF-α and IL-1β) increased 1 month AE (Figure 4) which corroborated with the mRNA levels of the inflammatory cytokines as reported previously [8]. Increase of conjunctival epithelium encoded goblet cell-specific MUC5AC at mRNA and protein levels were observed 1 month AE (Figure 4).

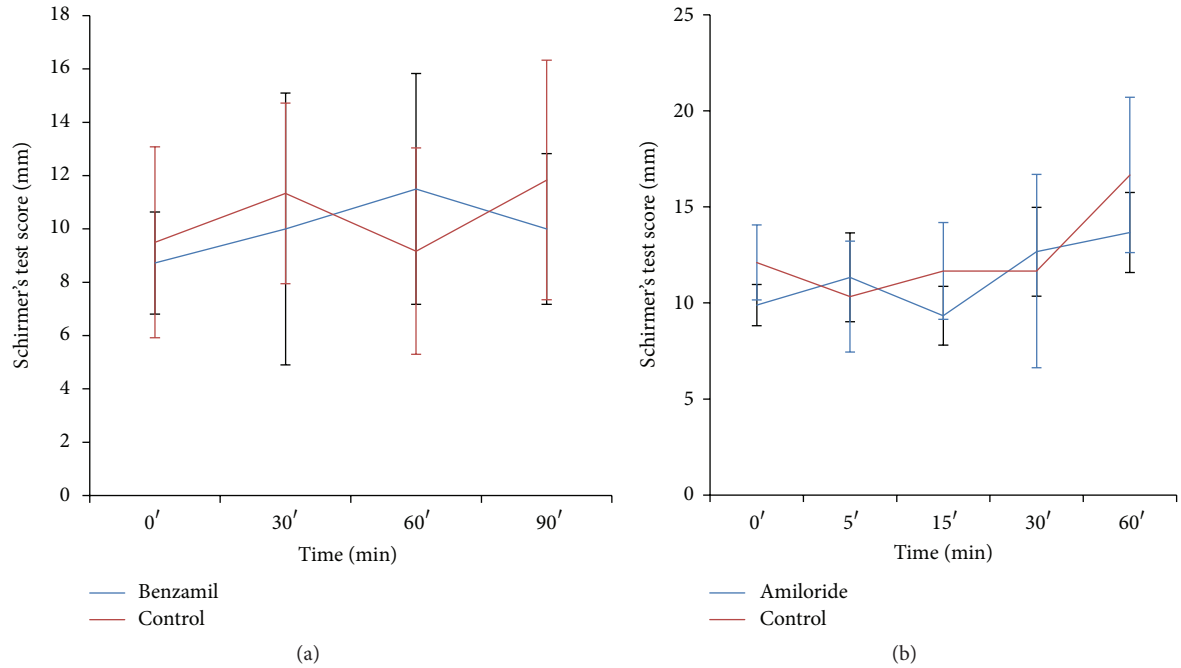

Figure 5: Effect of epithelial sodium channel blockers on rabbit DE model. The application of epithelial sodium channel blockers, benzamil (a) and amiloride (b), did not significantly increase the tear quantity in our rabbit DE model.

3.5. Effect of Amiloride and Benzamil Treatment on Conjunctiva Secretion. The two ENaC blockers did not increase tear secretion in our rabbit DE model as measured by SIIt (Figure 5).

3.6. Open-Circuit Potential Difference and Depolarization after Amiloride Treatment at the Ocular Surface of Rabbits. The PD measurements for the 10 seconds before the perfusion system was switched from PBS to amiloride channel were -272 ± 6 mV for rabbit eyes in the operated group ($n = 4$) and -159 ± 3 mV for the control group ($n = 4$). The difference in PDs was highly significant ($P < 0.005$). After the ocular surface was perfused with amiloride, the 10-second average PD reached -133 ± 4 mV in the operated eyes and -90 ± 4 mV in the control eyes. The magnitude of depolarization was statistically larger ($P < 0.05$) in the operated eyes than in the control eyes (Figure 6).

3.7. Statistical Correlations between Various Tests. Using Spearman correlation analysis, higher SIIt scores are closely associated with lower rose Bengal test scores (negatively correlated, correlation coefficient = −0.57, $P = 0.02$). Additionally, RT-qPCR of IL-1β and TNF-α were significantly correlated (correlation coefficient = 0.72, $P = 0.02$). The changes of inflammatory biomarkers did not correlate with that of the clinical tests (fluorescein staining, rose Bengal staining, and Schirmer tests).

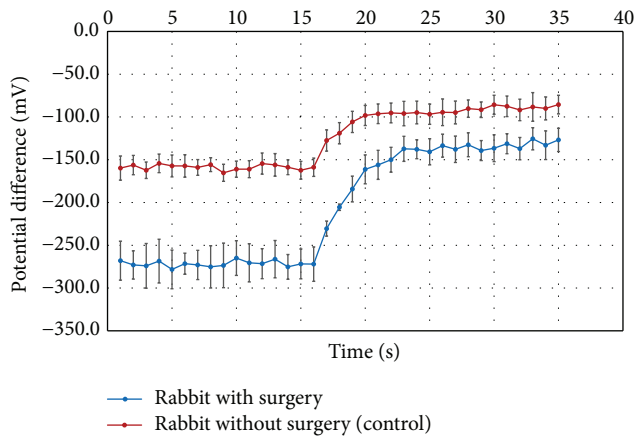

Figure 6: Potential difference recordings of the rabbit eyes after surgery compared to rabbit eyes without surgery. The potential differences were recorded for the rabbit eyes 5 months AE ($n = 4$) and control eyes ($n = 4$). The perfusion channel was switched from PBS to amiloride at 13 seconds, with 2-3 seconds required for the new solution to reach the ocular surface. Data are presented as mean Standard Error Method (SEM).

4. Discussion

In our study, as expected, the rabbits showed increased fluorescein and rose Bengal staining of the ocular surface 1 month AE, characteristic of DE phenotype. Interestingly, no significant reduction was found in tear secretion by Schirmer tests as compared to BE. Possible explanations as to why no significant reduction in tear secretion was seen after resection of the LG, HG, and NM have been extensively discussed in a separate publication [8].

It has been assumed that accessory LGs are responsible for the remaining tear secretion capacity in the absence of the

main LG [6, 15]. However, increasing evidence supports the notion that the conjunctiva can be an important contributor [5, 8, 16, 17]. The accessory LGs are embedded in the conjunctiva, and hence the surface area of conjunctiva is substantially larger than the sum of secreting acinar cell surface area of the accessory LGs. It is not unreasonable to assume that conjunctiva contributes substantial amount to the tear volume in the absence of main LG. Significant difference between SIt and SIIt scores in our rabbit model suggests that sensory regulation of the ocular surface plays an important role. Since accessory LGs have similar functions [18] and innervations as the main LG [19], they are assumed to be under identical reflex control [2]. Although a local transcellular osmotic mechanism is believed to govern the fluid and electrolyte transport [20] fluid secretion by the conjunctiva can also be stimulated [1]. The presence of parasympathetic nerves in rat conjunctiva [21] and increased conjunctiva Cl^- and fluid secretion by sympathomimetic agonists [22, 23] suggests that neural influence of conjunctiva secretion cannot be ruled out. And if so, local anesthesia of the secretory nerve terminals could also suppress the secretion output of the conjunctival epithelium. Differences between scores of SIt and SIIt in our study could reflect, at least to a large extent, the basal level tear secretion from accessory LGs and the conjunctiva, whereas it is very difficult, if not impossible, to determine the proportion of contributions from accessory LGs or conjunctiva to the remaining tear secretion capacity.

Contemporary clinical assessments of DE in animal models have certain shortcomings. Tear breakup time and corneal/conjunctiva staining are extremely difficult to evaluate objectively, especially in small animals. Schirmer tests results provide no direct evidence of ocular surface damage. Osmolarity test is expensive and has variable cutoffs [24]. In human, correlations between clinical symptoms, signs of DE, and diagnostic test results have been disappointing as well [25–29]. In our study, poor correlation among the clinical tests (fluorescein staining, rose Bengal staining, and Schirmer tests) is consistent with previous studies. Molecular biomarker based diagnostics, on the other hand, can offer a standardized, objective, and precise measurement of the status of ocular diseases [30] and should be used as adjuncts when possible.

DED associated ocular surface inflammation [31] is caused by increased level of inflammatory cytokines (IL-1, IL-6, TNF-α, and IL-17) in tear fluid, corneal/conjunctival epithelia, and increased infiltration of dendritic and T-cells in conjunctiva [32]. In our studies, removal of main LG, HG, and NM led to inflammatory responses at the ocular surface as depicted by increased mRNA [8] and protein levels of TNF-α and IL-1β. Rabbits with sham surgeries did not show significant increase in biomarker mRNA and protein (data not shown), suggesting that persistent elevation of these markers 1 month AE is not a direct result of surgical procedure itself. Although there was no significant change in tear production at 1 month AE, biomarker evaluations confirmed the increased inflammation which corroborated with the presence of DE phenotype at the ocular surface. Our data is consistent with Solomon et al. who demonstrated that DE is associated with increased production of proinflammatory cytokines (IL-1 and TNF-α) in conjunctiva [33]. To the best of our knowledge, overexpression of goblet cell-specific MUC5AC in response to acute DE condition created by surgery is a novel finding in our study. In association with the persistent normal level of tear secretion, MUC5AC overproduction likely contributed to the spontaneous recovery of ocular DE phenotype with time in our rabbit DE model [8]. Gilbard et al. noted reduced conjunctival goblet cell density in their rabbit DE model after cauterizing the LG excretory duct and surgically removing the NM and HG [17], whereas with mucin-specific staining, we were not able to discern any changes in the number or morphology of goblet cells in CIC specimens BE and AE [8]. The exact mechanisms of goblet cell mucin regulations in our rabbit DE model await further investigation.

We isolated both total RNA and protein from CIC specimens, a rapid, convenient, and minimally invasive technique to collect one to three layers of cells from bulbar conjunctival surface [34]. The CIC has been widely performed on subjects to confirm a variety of ocular surface diseases and monitor changes at conjunctival surface. Total RNA and protein isolated from CIC specimen detected subtle changes in mRNA and protein levels of the DED associated cytokines (TNF-α and IL-1β) and MUC5AC. Biomarkers provided objective and quantitative data that significantly enhanced the characterization of rabbit ocular surface pathology. One CIC specimen per eye at a specific time point offered sufficient high quality total RNA and protein for analyzing several genes without sacrificing the animals. This also enabled us to monitor these rabbits longitudinally and lowered experimental cost [8].

ENaC has been shown to be present in rabbit conjunctiva [35]. Shi and Candia concluded that the electrogenic Na^+ reabsorption across rabbit conjunctiva was amiloride-insensitive [36], indicating the important roles played by Na^+ dependent cotransporters such as those carrying glucose and amino acids in series with the basolaterally located Na^+-K^+ pump. Hara et al. recently demonstrated increased tear secretion as measured by Schirmer test after the application of amiloride at the rabbit ocular surface [37]. However, we were not able to reproduce their results in our rabbit model. Even using more potent ENaC inhibitor, benzamil [12], no significant increase in tear production was seen in the present study. We concluded that Schirmer test, given its large variation between measurements, may not be sensitive enough to detect subtle change in tear production. Therefore, we further assessed the baseline ocular PD and its response to the application of amiloride in rabbit eyes with and without surgery. Significantly higher (more negative) PD in the operated rabbit eyes was noted in comparison to eyes without surgery. Since electrogenic Cl^- secretion and Na^+ reabsorption across superficial cell apical membrane of the corneal and conjunctival epithelia contribute to the PD [13], the ocular surface tissues must have reached a new equilibrium of higher Cl^- secretion and/or Na^+ reabsorption. Higher magnitude of PD depolarization in the operated eyes in response to the application of amiloride indicates the presence of an elevated amiloride-sensitive Na^+ conductance (reabsorption) across the epithelia. Although amiloride-insensitive higher Na^+

reabsorption mechanism could not be measured in the study, it presumably exists. Likewise, a higher Cl^- conductance (secretion) most probably is present as well. Our PD measurements demonstrate electrophysiological support of higher tear output across the ocular surface in rabbit eyes without LG, HG, and NM.

To summarize, in this rabbit DED model, although Schirmer tests were unchanged BE and AE, analysis of biomarkers corroborated with the clinical examination findings and confirmed the development of DE condition. Assessing DED pertinent biomarkers enhanced the results obtained from standard clinical tests and is a valuable addition to the tools of ocular surface evaluation. It was interesting to note the elevated MUC5AC expression in the acute DE condition created by surgery but its mechanism requires further investigation. No measurable increased tear secretion was detected with Schirmer test with topical application of amiloride in rabbit eyes AE. However, the open-circuit PD measurement provided a sensitive modality to detect the underlying electrophysiological changes at the rabbit ocular surface AE.

Competing Interests

The authors declare that they have no conflict of interests.

Authors' Contributions

Yuan Ning and Dhruva Bhattacharya contributed equally.

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References

[1] D. A. Dartt, "Regulation of mucin and fluid secretion by conjunctival epithelial cells," *Progress in Retinal and Eye Research*, vol. 21, no. 6, pp. 555–576, 2002.

[2] "The definition and classification of dry eye disease: Report of the definition and classification subcommittee of the international Dry Eye WorkShop (2007)," *Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[3] L. Thörig, E. J. Van Agtmaal, E. Glasius, K. L. Tan, and N. J. Van Haeringen, "Comparison of tears and lacrimal gland fluid in the rabbit and guinea pig," *Current Eye Research*, vol. 4, no. 8, pp. 913–920, 1985.

[4] D. Y. Maitchouk, R. W. Beuerman, T. Ohta, M. Stern, and R. J. Varnell, "Tear production after unilateral removal of the main lacrimal gland in squirrel monkeys," *Archives of Ophthalmology*, vol. 118, no. 2, pp. 246–252, 2000.

[5] A. Hornblass, C. Guberina, and B. J. Herschorn, "Palpebral dacryoadenectomy for epiphora," *Ophthalmic Plastic and Reconstructive Surgery*, vol. 4, no. 4, pp. 227–230, 1988.

[6] W. Scherz and C. H. Dohlman, "Is the lacrimal gland dispensable? Keratoconjunctivitis sicca after lacrimal gland removal," *Archives of Ophthalmology*, vol. 93, no. 4, pp. 281–283, 1975.

[7] M. A. Watsky, M. M. Jablonski, and H. F. Edelhauser, "Comparison of conjunctival and corneal surface areas in rabbit and human," *Current Eye Research*, vol. 7, no. 5, pp. 483–486, 1988.

[8] D. Bhattacharya, Y. Ning, F. Zhao et al., "Tear production after bilateral main lacrimal gland resection in rabbits," *Investigative Opthalmology & Visual Science*, vol. 56, no. 13, pp. 7774–7783, 2015.

[9] Z.-Y. Chen, Q.-F. Liang, and G.-Y. Yu, "Establishment of a rabbit model for keratoconjunctivitis sicca," *Cornea*, vol. 30, no. 9, pp. 1024–1029, 2011.

[10] N. Li, X. Deng, Y. Gao, S. Zhang, M. He, and D. Zhao, "Establishment of the mild, moderate and severe dry eye models using three methods in rabbits," *BMC Ophthalmology*, vol. 13, no. 1, article 50, 2013.

[11] T. Nakamura, Y. Hata, M. Nagata et al., "JBP485 promotes tear and mucin secretion in ocular surface epithelia," *Scientific Reports*, vol. 5, Article ID 10248, 2015.

[12] A. J. Hirsh, J. R. Sabater, A. Zamurs et al., "Evaluation of second generation amiloride analogs as therapy for cystic fibrosis lung disease," *Journal of Pharmacology and Experimental Therapeutics*, vol. 311, no. 3, pp. 929–938, 2004.

[13] M. H. Levin and A. S. Verkman, "Aquaporins and CFTR in ocular epithelial fluid transport," *Journal of Membrane Biology*, vol. 210, no. 2, pp. 105–115, 2006.

[14] M. H. Levin and A. S. Verkman, "CFTR-regulated chloride transport at the ocular surface in living mice measured by potential differences," *Investigative Ophthalmology and Visual Science*, vol. 46, no. 4, pp. 1428–1434, 2005.

[15] J. P. Gilbard, S. R. Rossi, K. G. Heyda, and D. A. Dartt, "Stimulation of tear secretion by topical agents that increase cyclic nucleotide levels," *Investigative Ophthalmology and Visual Science*, vol. 31, no. 7, pp. 1381–1388, 1990.

[16] M. H. I. Shiue, A. A. Kulkarni, H. J. Gukasyan, J. B. Swisher, K.-J. Kim, and V. H. L. Lee, "Pharmacological modulation of fluid secretion in the pigmented rabbit conjunctiva," *Life Sciences*, vol. 66, no. 7, pp. PL105–PL111, 2000.

[17] J. P. Gilbard, S. R. Rossi, and K. L. Gray, "A new rabbit model for keratoconjunctivitis sicca," *Investigative Ophthalmology and Visual Science*, vol. 28, no. 2, pp. 225–228, 1987.

[18] J. L. Ubels, I. K. Gipson, S. J. Spurr-Michaud, A. S. Tisdale, R. E. Van Dyken, and M. P. Hatton, "Gene expression in human accessory lacrimal glands of Wolfring," *Investigative Ophthalmology and Visual Science*, vol. 53, no. 11, pp. 6738–6747, 2012.

[19] P. Seifert, S. Stuppi, and M. Spitznas, "Distribution pattern of nervous tissue and peptidergic nerve fibers in accessory lacrimal glands," *Current Eye Research*, vol. 16, no. 4, pp. 298–302, 1997.

[20] O. A. Candia and L. J. Alvarez, "Fluid transport phenomena in ocular epithelia," *Progress in Retinal and Eye Research*, vol. 27, no. 2, pp. 197–212, 2008.

[21] J. D. Ríos, D. Zoukhri, I. M. Rawe, R. R. Hodges, J. D. Zieske, and D. A. Dartt, "Immunolocalization of muscarinic and VIP receptor subtypes and their role in stimulating goblet cell secretion," *Investigative Ophthalmology and Visual Science*, vol. 40, no. 6, pp. 1102–1111, 1999.

[22] H. C. Turner, L. J. Alvarez, and O. A. Candia, "Cyclic AMP-dependent stimulation of basolateral K^+ conductance in the rabbit conjunctival epithelium," *Experimental Eye Research*, vol. 70, no. 3, pp. 295–305, 2000.

[23] U. B. Kompella, K.-J. Kim, M. H. I. Shiue, and V. H. L. Lee, "Cyclic AMP modulation of active ion transport in the pigmented rabbit conjunctiva," *Journal of Ocular Pharmacology and Therapeutics*, vol. 12, no. 3, pp. 281–287, 1996.

[24] M. Li, C. Du, D. Zhu, M. Shen, L. Cui, and J. Wang, "Daytime variations of tear osmolarity and tear meniscus volume," *Eye and Contact Lens*, vol. 38, no. 5, pp. 282–287, 2012.

[25] B. Caffery, R. L. Chalmers, H. Marsden et al., "Correlation of tear osmolarity and dry eye symptoms in convention attendees," *Optometry and Vision Science*, vol. 91, no. 2, pp. 142–149, 2014.

[26] M. A. Lemp, A. J. Bron, C. Baudouin et al., "Tear osmolarity in the diagnosis and management of dry eye disease," *The American Journal of Ophthalmology*, vol. 151, no. 5, pp. 792–798.e1, 2011.

[27] B. Sullivan, "Challenges in using signs and symptoms to evaluate new biomarkers of dry eye disease," *Ocular Surface*, vol. 12, no. 1, pp. 2–9, 2014.

[28] B. D. Sullivan, L. A. Crews, E. M. Messmer et al., "Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications," *Acta Ophthalmologica*, vol. 92, no. 2, pp. 161–166, 2014.

[29] F. Amparo, Y. Jin, P. Hamrah, D. A. Schaumberg, and R. Dana, "What is the value of incorporating tear osmolarity measurement in assessing patient response to therapy in dry eye disease?" *American Journal of Ophthalmology*, vol. 157, no. 1, pp. 69–77.e2, 2014.

[30] G. N. Foulks and S. C. Pflugfelder, "New testing options for diagnosing and grading dry eye disease," *American Journal of Ophthalmology*, vol. 157, no. 6, pp. 1122–1129, 2014.

[31] W. Stevenson, S. K. Chauhan, and R. Dana, "Dry eye disease: an immune-mediated ocular surface disorder," *Archives of Ophthalmology*, vol. 130, no. 1, pp. 90–100, 2012.

[32] C. S. De Paiva, S. Chotikavanich, S. B. Pangelinan et al., "IL-17 disrupts corneal barrier following desiccating stress," *Mucosal Immunology*, vol. 2, no. 3, pp. 243–253, 2009.

[33] A. Solomon, D. Dursun, Z. Liu, Y. Xie, A. Macri, and S. C. Pflugfelder, "Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease," *Investigative Ophthalmology and Visual Science*, vol. 42, no. 10, pp. 2283–2292, 2001.

[34] M. Mrugacz, I. Kasacka, A. Bakunowicz-Lazarczyk, M. Kaczmarski, and W. Kulak, "Impression cytology of the conjunctival epithelial cells in patients with cystic fibrosis," *Eye*, vol. 22, no. 9, pp. 1137–1140, 2008.

[35] U. B. Kompella, K.-J. Kim, and V. H. L. Lee, "Active chloride transport in the pigmented rabbit conjunctiva," *Current Eye Research*, vol. 12, no. 12, pp. 1041–1048, 1993.

[36] X.-P. Shi and O. A. Candia, "Active sodium and chloride transport across the isolated rabbit conjunctiva," *Current Eye Research*, vol. 14, no. 10, pp. 927–935, 1995.

[37] S. Hara, A. Hazama, M. Miyake et al., "The effect of topical amiloride eye drops on tear quantity in rabbits," *Molecular Vision*, vol. 16, pp. 2279–2285, 2010.

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Review Article

Comprehensive Review of the Literature on Existing Punctal Plugs for the Management of Dry Eye Disease

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Numerous designs of punctal and canalicular plugs are available on the market. This variety presents challenges to ophthalmologists when choosing punctal plugs for the management of various ocular conditions. The aim of this literature review is to provide a classification system for lacrimal occlusive devices based on their location and duration of action as well as to identify different characteristics of each one of them. We want to give a comprehensive overview on punctal and canalicular plugs including their manufacturing companies, indications, and complications that have been reported in various articles. PubMed and Google Scholar were used to identify articles written in English as well as few articles written in Japanese, Chinese, Slovak, and Spanish that had abstracts in English. Nine different companies that manufacture punctal and canalicular plugs were identified and their plugs were included in this review. Punctal and canalicular plugs are used in the management of various ocular conditions including dry eye disease and punctal stenosis as well as in ocular drug delivery. Although they are a relatively safe option, associated complications have been reported in the literature such as infection, allergic reaction, extrusion, and migration.

1. Introduction

Dry eye is a condition commonly seen by eye care practitioners; as many as 25% of patients seen in clinic have symptoms of dry eye [1]. The International Dry Eye Workshop (DEWS) defines dry eye as a multifactorial disease of tears and ocular surface with symptoms of visual disturbance, discomfort, and tear film instability with associated ocular inflammation and increased tear film osmolarity [2]. Data from Women's Health Studies (WHS) and Physicians' Health Studies (PHS) estimates 3.2 million women and 1.6 million men aged 50 years or older in the United States suffering from moderate to severe dry eye [3–5]. It is estimated that 8.5 million Americans spend more than 300 million dollars on artificial tear preparations and other related over-the-counter medications for dry eye disease [6]. The DEWS classified dry eye disease into four levels depending on severity of the disease and treatment options were recommended accordingly [7]. Topical lubricants, topical cyclosporine (Restasis), tetracyclines, and punctal plugs are a few of the available treatment options [8]. Plugs can be classified according to their location (punctal versus canalicular) and their duration of placement (temporary versus permanent). They are made of different materials that include collagen, silicone, hydrogel, polydioxanone, and acrylic. The ability to preserve tears makes them useful in certain cases of refractive surgery and contact lens intolerance [9]. Lacrimal occlusion with plugs prolongs the effects of lubricants and preserves natural tears. They are relatively contraindicated in patients with dry eyes and coexisting inflammation. Blocking the puncta exposes the ocular surface to tears having preexisting proinflammatory cytokines that worsen the ocular inflammation [10].

The use of punctal plugs is not limited to dry eye disease. Perforated punctal plugs have been successfully utilized in the treatment of punctal stenosis resulting in significant improvement in epiphora associated with the stenosis. Punctal plugs can be used for ocular drug delivery and can modulate the effect of other forms of topical treatment [11, 12]. This

can be utilized in the treatment of glaucoma by increasing the drug retention time [9]. Both punctal and canalicular plugs have been associated with complications that have been reported in the literature. They can result in infections such as canaliculitis, biofilm formation, extrusion, migration, epiphora, and chronic irritation [9].

The purpose of this literature review is to give clinicians an update on different types of punctal and canalicular plugs, with recent advancements in designs and techniques. Choosing the best suitable punctal/canalicular plug for treatment of various ocular surface disorders (dry eye disease, punctal stenosis, epithelial erosions, and ocular drug delivery) may be difficult for clinicians as a large variety of punctal and canalicular plugs in different shapes, designs, and materials are available. It is important for the clinicians to be familiar with the complications that have been reported with different lacrimal plugs and to evaluate patients for any preexisting ocular or lid abnormalities. This paper provides a comprehensive overview of all the available punctal and canalicular plugs and can serve as a guide for clinicians to choose the most suitable lacrimal plug when treating the above-mentioned conditions.

2. Materials and Methods

PubMed and Google Scholar were searched for studies published up to October 2015. Eligibility criteria included studies evaluating indications, contraindications, adverse effects, shapes, designs, and characteristics of different punctal and canalicular plugs. Using Google, we searched for different manufacturers of punctal and canalicular plugs and used pictures (after getting permission from respective manufacturers) and characteristic features of the plugs to compile classification tables. We also evaluated different types of punctal and canalicular plugs microscopically to evaluate their characteristics. Keywords included punctal plugs, dry eyes, punctal stenosis, silicone plugs, perforated plugs, drug delivery, collagen plugs, SmartPlug, EagleFlex, Lacrimal Gland Occlusion, and intracanalicular plugs. A variety of articles related to punctal plugs were included in this review.

2.1. Classification of Punctal and Canalicular Plugs. Lacrimal occlusive devices can be classified into punctal and canalicular plugs (Figure 1). Freeman, in 1975, developed the dumbbell shaped punctal plug made of silicone and this concept of punctal plug is still in use [13]. Punctal plugs rest at the punctal opening making them easily visible and, hence, are removable without much difficulty. In contrast, canalicular plugs are not visible as they are placed inside the canaliculus (either the vertical or the horizontal canaliculus), making extrusion unlikely but increasing the risk of migration and difficulty in localizing their position without ultrasound [14]. Occlusion of the lacrimal drainage system with temporary or permanent plugs is a widely used nonpharmacological therapy for conserving tears. A wide variety of lacrimal plugs with specific indications are in use. Both horizontal and vertical canalicular plugs can be further classified into temporary and permanent. Temporary short duration canalicular plugs (Figure 2(a)) are used before attempting extended duration or permanent occlusion to assess risk of epiphora and the probability of symptomatic relief [15]. Temporary short duration ones plugs are usually made of animal collagen and last for 4–14 days. Temporary extended duration plugs (Figure 2(b)) are used following refractive surgery, for dry eye disease and for ocular retention of medications [16]. Temporary extended duration plugs can last from 2 to 6 months [17]. They are made of different materials such as glycolic acid with trimethylene carbonate, E-Caprolactone-L-Lactide copolymer (PCL), and polydioxanone (PDS).

2.2. Characteristics of Punctal Plugs. Different designs and shapes of plugs have been developed to increase their effectiveness and to minimize complications. Generally punctal plugs have a head on the top and are shaped like an umbrella. The head facilitates removal of the plug if necessary [26]. They usually have a slender neck and a cone-shaped thicker base. The majority of the punctal plugs are made of silicone, but teflon, hydroxyethylmethacrylate (HEMA), and polymethylmethacrylate (PMMA) have been tested [9]. We evaluated different punctal plugs under the microscope and classified them based on their shapes (Table 1). Punctal plugs have different shaft designs (e.g., tapered shafts and straight shafts) with pros and cons of different styles. The head portion can have reservoirs in some designs for increased trapping of tears. There are variations in the collarette such as a slanted collarette, which improves the fit. Some designs have tractional ribs for greater flexibility while some have collapsible noses that spring open once inside the puncta (Table 1). Perforated punctal plugs have a central lumen; they are used in treating punctal stenosis and partial occlusion by allowing some tear flow through the plug [27]. Punctal plug manufacturers, sizes, and characteristics are discussed in Table 2.

2.3. Characteristics of Canalicular Plugs. Canalicular plugs for temporary use are usually rod-shaped and available in different sizes and colors depending on the punctal size. They are inserted into the canaliculus making it difficult to visualize them or monitor their position. To achieve complete occlusion of the lacrimal drainage system, the diameter of the plug is more important than its length [9]. Special designs for permanent use have been developed. The Form Fit plug (Figure 2(c)) is a vertical canalicular plug made of hydrogel that expands into a soft gelatinous material after contact with the tear film, filling and conforming to the shape of the vertical canaliculus [17]. Thermosensitive acrylic canalicular plugs (Medennium SmartPlug) have been in use since 2002. They become shorter and thicker at body temperature. The plug has a diameter of 0.4 mm and length of 9 mm before insertion that change after insertion to a diameter of 1 mm and a length of 2 mm [28]. Newer materials are thought to reduce bacterial adhesion and chances of infections [9]. Horizontal canalicular plugs can be temporary or permanent (Figure 3). Herrick canalicular plugs, made of silicone, are placed in the horizontal canaliculus and shaped like a golf tee. They do not require punctal dilation prior to insertion. Removal of these horizontal canalicular plugs may prove challenging, in some

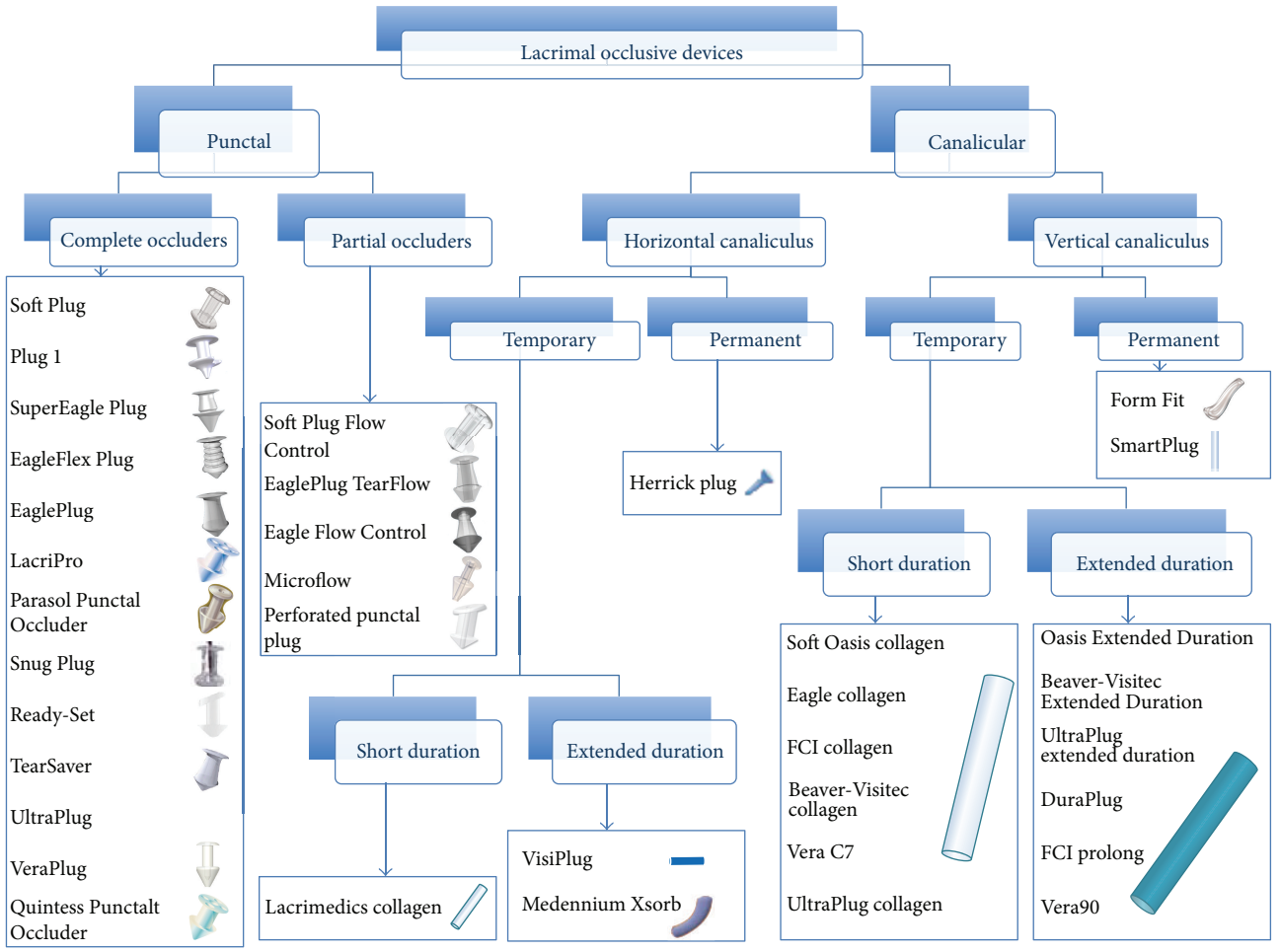


Figure 1: Classification of lacrimal occlusive devices based on shape, location, and duration of action.

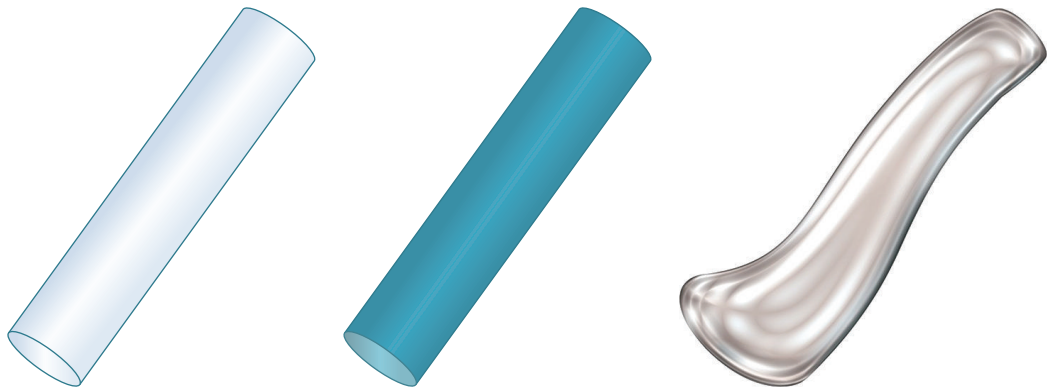


Figure 2: Vertical canalicular plugs. (a) Schematic of the temporary short duration plug made of collagen and effective for 4–14 days. (b) Representation of the temporary extended duration plug made of different materials (polydioxanone, glycolic acid and trimethylene carbonate, and E-Caprolactone-L-Lactide copolymer) and effective for about 2–6 months depending on the manufacturer. (c) Schematic of the hydrogel (Form Fit) plug that expands with hydration to mold into canaliculus and be permanently effective (*image C courtesy of* http://www.oasismedical.com).

Table 1: Shapes of punctal plugs. Several designs are made by different companies to increase the effectiveness of punctal plugs with different shapes of the shafts, collapsible noses, reservoirs, and traction ribs.

Design	Advantages	Name	Model
Tapered shaft	Designs extra force horizontally to keep plug in place.	EaglePlug	
		TearSaver	
		SuperEagle Plug	
Collapsible nose	Collapsible hollow nose adheres the plug to the shape of ampulla and springs open once inside the puncta.	Parasol Punctal Occluder	
Reservoired head	Indentations trap tears and minimize foreign body sensation.	LacriPro	
		Quintess Punctal Occluder	
Ribbed shaft	Greater flexibility.	EagleFlex Plug	

Table 1: Continued.

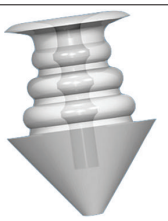
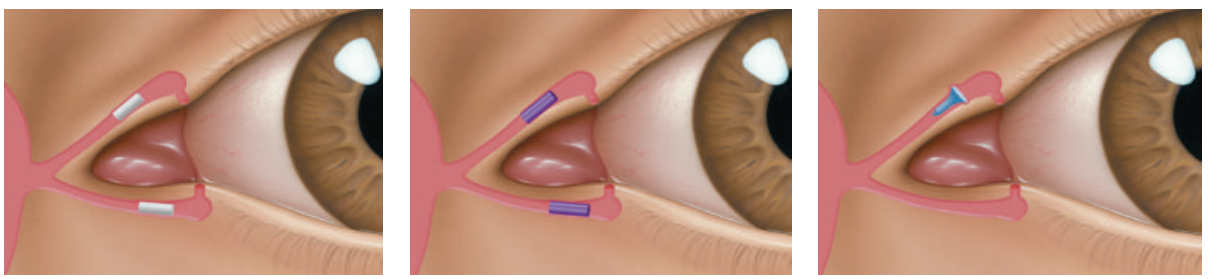
Design	Advantages	Name	Model
		SuperFlex	
Perforated shaft	Slanted collarette perforated lumen.	Soft Plug Flow Control	
		Eagle Flow Control	
		EaglePlug TearFlow	
		Microflow	
		Perforated punctal plug	
Slanted lip	Resists migration and prevents rubout. Conforms to the natural anatomy of eyelid.	Ready-Set	

Table 1: Continued.

Design	Advantages	Name	Model
Dual lobe tip	Fits a range of punctal sizes.	Plug 1	
Stretched shaft	Returns to natural shape after insertion.	Snug Plug	
Straight shaft	Low profile dome and easy insertion.	VeraPlug	
		Parasol Punctal Occluder	
		Soft Plug	
		UltraPlug	



(a) (b) (c)

Figure 3: Horizontal canalicular plugs. (a) Collagen plug (Lacrimedics) that is meant to last for about two weeks. (b) Temporary extended duration plug (VisiPlug by Lacrimedics) made of polydioxanone does not swell with moisture and lasts about 6 months. (c) Permanent canalicular plug (Herrick plug by Lacrimedics) is shaped like a golf tee (*photos courtesy of* http://www.lacrimedics.com).

Table 2: Punctal plugs [18–25] (types of punctal plugs, manufacturers, and characteristics discussed in detail).

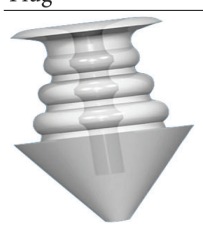
Name	Size	Material	Characteristics	Manufacturer
 Soft Plug	5 sizes (0.4–0.8 mm) (micro, mini, petite, small, and medium)	Medical grade silicone	Pointed nose simplifies insertion; large anchor secures plug.	Oasis
 Plug 1	1 size (fits 0.5–0.8 mm puncta)	Silicone	Dual lobed design to fit a range of punctal sizes.	EagleVision
 SuperEagle Plug	Small (0.4–0.6 mm) Medium (0.6–0.8 mm) Large (>0.8 mm) Values = puncta size	Low durometer silicone	Low durometer silicone with low profile rim for more comfort. Tapered shape and wide flexed nose design with a goal of good retention.	EagleVision
 SuperFlex Plug	11 sizes (combinations with 0.3–1.3 mm diameter, 1.1–2.0 mm length)	Silicone	Better fit with good retention. Easy insertion.	EagleVision

Table 2: Continued.

Name	Size	Material	Characteristics	Manufacturer
 EaglePlug	5 sizes (0.4–0.8 mm)	Silicone	Tapered shaft exerts horizontal force to keep plug in place. Easy insertion and removal.	EagleVision
 EagleFlex Plug	6 sizes (0.4–0.9 mm)	Silicone	Tapered shaft with traction ribs for better retention and flexibility. External ribs give 30% more surface area. Thin rim decreases corneal contact.	EagleVision
 LacriPro Punctum	4 sizes (0.3, 0.5, 0.7, and 0.9 mm) (X-small, small, medium, and large)	Medical grade silicone	Reservoir indentations trap tears and decrease foreign body sensation. The number of indentations corresponds to the size of the plug.	Lacrimedics
 Parasol Punctal Occluder	X-small (0.25–0.35 mm) Small (0.35–0.65 mm) Medium (0.6–0.85 mm) Large (>0.9 mm) Values = puncta size	Silicone	Hollow nose. Umbrella-like head which squeezes as it is inserted and springs open once in place. No need for punctal dilation.	Beaver-Visitec
 Snug Plug	1 size fits all	Silicone	Preloaded in a stretched position and returns to natural shape after insertion (Figure 4).	FCI

Table 2: Continued.

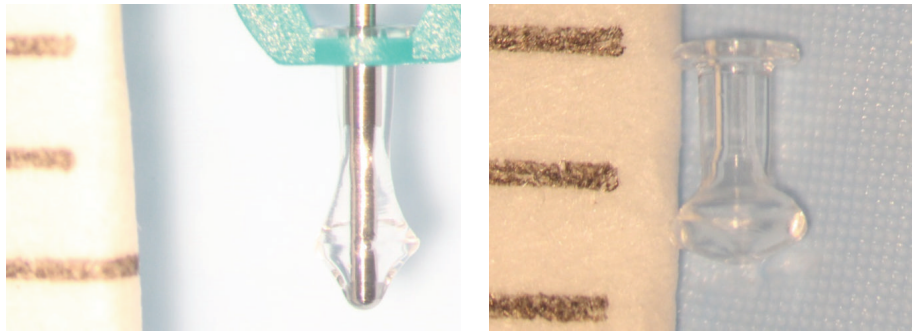
Name	Size	Material	Characteristics	Manufacturer
Ready-Set	7 sizes (0.4–1.0 mm) (slim mini, slim petite, micro, mini, small, medium, and large)	Silicone	Slanted lip in sizes 0.6 mm and larger makes it a better fit. Conforms to natural anatomy of the eyelid. Sizes 0.6 mm and larger also have slightly larger bulbs to resist migration and prevent rubout.	FCI
 TearSaver	5 sizes (0.4–0.8 mm)	Silicone	Tapered shaft exerts extra horizontal force to keep plug in place.	FCI
UltraPlug	5 sizes (0.4–0.8 mm)	Silicone	Straight shaft. Low profile cap design minimizes foreign body sensation.	Surgical Specialties Corporation
 VeraPlug	Small (0.4–0.6 mm) Medium (0.6–0.7 mm) Large (0.7–0.8 mm) X-Large (0.8–1.0 mm) Values = puncta size	Silicone	Low profile dome for more comfort. Proproprietary shaft design for easy insertion and good fit (Figure 5).	Lacrivera
 Quintess Punctal Occluder (AquaFlo)	4 sizes (0.3, 0.5, 0.7, 0.9 mm) (X-small, small, medium, and large)	Silicone	Reservoir indentations are designed to decrease foreign body sensation. Indentations correspond to plug size (Figure 5).	AlphaMed

Table 2: Continued.

Name	Size	Material	Characteristics	Manufacturer
 Soft Plug Flow Control	3 sizes (0.6–0.8 mm)	Soft silicone	Partial occluder. Opening through nose portion for partial occlusion. Low profile dome head. Softer, flexible silicone makes it more comfortable. Pointed nose secures it firmly. Patent lumen allows for use in punctal stenosis.	Oasis
 Eagle Flow Controller	4 sizes (0.5–0.8 mm)	Silicone	Tapered shaft. Partial occluder. Patent lumen allows for use in punctal stenosis. Tapered shaft increases vector force to keep the plug in position.	EagleVision
 EaglePlug TearFlow	6 sizes: 0.5 mm plug, 0.2 mm lumen 0.6 mm plug, 0.3 mm lumen 0.7 mm plug, 0.3 mm lumen 0.8 mm plug, 0.3 mm lumen 0.9 mm plug, 0.4 mm lumen 1.0 mm plug, 0.5 mm lumen	Silicone	Partial occluder. Lumen size is 150% larger than Eagle Flow Control plug allowing for increased tear flow. Tapered shaft gives more controlled retention. Patent lumen allows for use in punctal stenosis.	EagleVision
 Microflow	Small (0.4–0.55 mm) Medium (0.55–0.7 mm) Large (0.7–0.85 mm) Values = punctal size	Silicone	Partial occluder. Patent lumen allows for use in punctal stenosis.	Beaver-Visitec

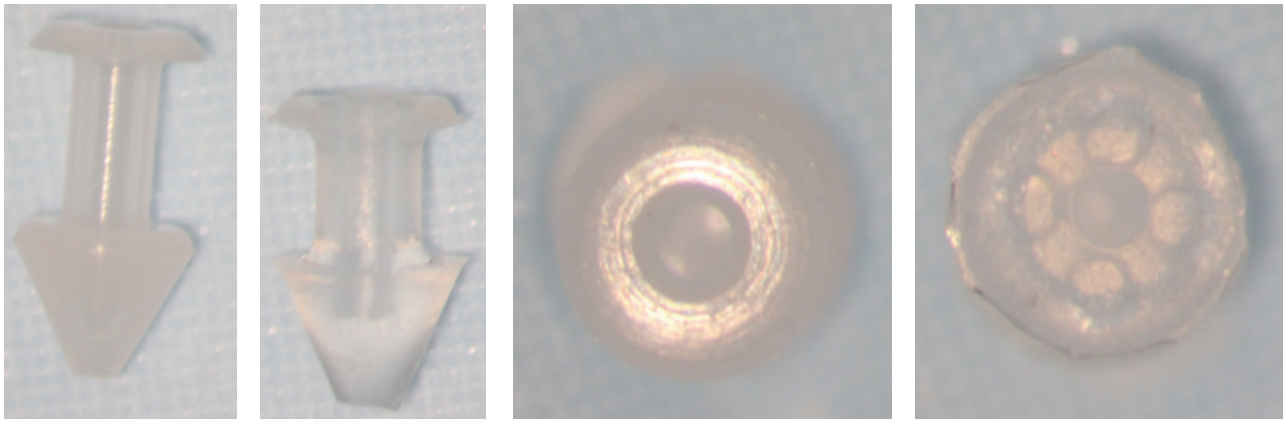
Table 2: Continued.

Name	Size	Material	Characteristics	Manufacturer
 Perforated punctal plug	2 sizes (0.7, 0.9 mm) (mini, medium)	Silicone coated with PVP (polyvinylpyrrolidone)	Partial occluder. Slanted collarette. PVP (polyvinylpyrrolidone) is hydrophobic and allows tear to flow smoothly through the perforation. Used in treating punctal stenosis.	FCI



(a) (b)

Figure 4: Snug Plug (FCI) is a punctal plug that is preloaded in a stretched position (a) compared to its natural shape (b) after release from inserter. The plug is on stretch for insertion with the goal of eliminating the step of punctal dilation prior to insertion. The widened bulb at the plug's base in the natural position acts to prevent the plug from falling out spontaneously. Each dash in scale represents 1 mm.



(a) (b)

Figure 5: Punctal plug without reservoirs (VeraPlug) on (a) compared with punctal plug with reservoirs (Quintess Punctal Occluder) on (b). The Quintess Punctal Occluder was designed to have reservoir indentations in the collar to trap tears.

cases, due to their intracanalicular location [29]. The Herrick plug is partially radiopaque and dyed blue in color to make localization possible with transillumination [9]. Horizontal and vertical canalicular plugs are discussed in Tables 3 and 4.

2.4. Indications of Punctal and Canalicular Plugs

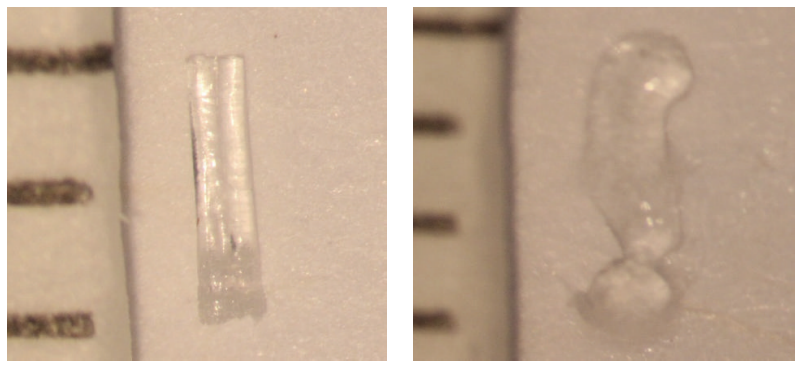
(i) Dry Eye Disease. Occlusion of the lacrimal drainage system with plugs is considered an option in patients with moderate dry eye syndrome. An article by the American Academy of Ophthalmology reviewed literature to assess efficacy and safety of punctal and canalicular plugs for treatment of dry eye disease. The use of lacrimal plugs improved the symptoms, enhanced the ocular surface health, and decreased the use of lubricants in dry eye disease [30]. Recently a survey was sent to researchers and expert ophthalmologists in order to identify the common treatments used for managing dry eye disease [31]. It was observed that topical therapies are most commonly prescribed including steroids, cyclosporine A, and autologous serum. Among the nontopical therapies, respondents commonly use punctal plugs, tetracycline, flaxseed supplements, and essential fatty acid supplements. In another study, 86% patients were free of symptoms of dry eye at 6-month follow-up and 76% of patients had stopped using lubricants after punctal occlusion with silicone punctal plugs [32]. Punctal occlusion is effective in treating many conditions that cause dry eyes such as Stevens-Johnson syndrome, keratoconjunctivitis sicca, contact lens wear, and superior limbic keratoconjunctivitis [33]. Another study evaluated canalicular occlusion with collagen and silicone plugs (Herrick plugs) in patients with dry eye related conjunctivitis. It was a prospective, randomized trial and at the 8-week visit, there was a marked reduction in total dry eye (94.2%) and conjunctival symptom scores (93%) which was in sharp contrast to the sham group that experienced no change from the baseline [34]. Silicone punctal plugs have been associated with a significant decrease in tear film osmolality and a 75% decrease in rose bengal staining in 17 patients with dry eye [35]. Silicone punctal plugs used in keratoconjunctivitis sicca patients showed an improvement in goblet cell density, tear film stability, and ocular staining scores [36]. In another study, both collagen and silicone plugs resulted in an increase

Table 3: Vertical canalicular plugs [18–25] (types of vertical canalicular plugs, manufacturers, and characteristics discussed in detail).

Name	Size	Material	Temporary/ permanent	Characteristics	Manufacturer
Form Fit	1 size (diameter 0.3 mm, length 3 mm)	Hydrogel	Permanent	Hydrates over 10 minutes after insertion and expands (Figure 6). With hydration it increases in size till it completely fills vertical canalicular cavity. It has low extrusion rate.	Oasis
SmartPlug	1 size (diameter 0.4 mm, length 6 mm)	Thermosensitive acrylic material	Permanent	Adjusts to shape and size of punctum. It shrinks to 1 mm after insertion to make it more comfortable by eliminating foreign body sensation. Less chance of extrusion. Can be flushed out with irrigation.	Medennium
Oasis Soft Plug Extended Duration	4 sizes (0.2–0.5 mm)	Glycolic acid and trimethylene carbonate	Temporary—extended duration	Effective up to 3 months (Figure 7(a)).	Oasis
DuraPlug	3 sizes (0.2–0.4 mm)	PCL (E-Caprolactone-L-Lactide copolymer)	Temporary—extended duration	Lasts 2–6 months.	EagleVision
Beaver-Visitec Extended Duration	4 sizes (0.2–0.5 mm)	Glycolic acid and trimethylene carbonate	Temporary—extended duration	Dyed with D&C Green. Lasts up to 3 months.	Beaver-Visitec
ProLong	3 sizes (0.3–0.5 mm)	Glycolic acid and trimethylene carbonate	Temporary—extended duration	Dyed with D&C Green number 6. Lasts up to 3 months.	FCI
Vera90	3 sizes (0.2–0.4 mm)	PCL (E-Caprolactone-L-Lactide copolymer)	Temporary—extended duration	Dyed violet with D&C Violet number 20 and is coated with calcium stearate (a noncollagenous and nonantigenic coating). Lasts up to 3 months (Figure 7(b)).	Lacrivera
UltraPlug Extended Wear	3 sizes (0.2–0.4 mm)	PCL (E-Caprolactone-L-Lactide copolymer)	Temporary—extended duration	Effective for 2–6-month duration.	Surgical Specialties Corporation
Oasis Soft Plug Collagen	3 sizes (0.2–0.4 mm) 2 mm length.	Collagen	Temporary—short duration	Lasts 2–5 days (see Figure 8(a)).	Oasis
Eagle collagen	3 sizes (0.2–0.4 mm)	Collagen	Temporary—short duration	Effective for 3–5 days and lasts for 7–10 days. Expands in punctum after insertion.	EagleVision
Beaver-Visitec collagen plug	3 sizes (0.2–0.4 mm)	Collagen	Temporary—short duration	Lasts 7–10 days.	Beaver-Visitec
FCI collagen plug	3 sizes (0.2–0.4 mm)	Collagen	Temporary—short duration	Lasts 5–7 days.	FCI
Vera C7	3 sizes (0.2–0.4 mm)	Collagen	Temporary—short duration	Effective for 7–10 days (Figure 8(b)).	Lacrivera
UltraPlug collagen	3 sizes (0.2–0.4 mm)	Collagen	Temporary—short duration	Effective for 10–14 days.	Surgical Specialties Corporation

Table 4: Horizontal canalicular plugs [18–25] (types of vertical canalicular plugs, manufacturers, and characteristics discussed in detail).

Name	Size	Material	Temporary/ permanent	Characteristics	Manufacturer
Herrick plug	3 sizes (0.3, 0.5, and 0.7 mm)	Medical grade silicone	Permanent	Shape of a golf tee and radiopaque. More comfortable initially but, because of the stagnant column of tear fluid between the plug and punctal opening, are theoretically more prone to infection. It has a collapsible bell design, which makes insertion easier.	Lacrimedics
VisiPlug	2 sizes (0.4–0.5 mm)	Polydioxanone (PDS)	Temporary—extended duration	Does not swell upon coming in contact with moisture (Figure 7(c)). Lasts up to 6 months.	Lacrimedics
Xsorb Plug	3 sizes (0.3–0.5 mm)	Glycolic acid and trimethylene carbonate	Temporary—extended duration	Dyed with D&C Green number 6. Lasts for about 3 months.	Medennium
Lacrimedics collagen	3 sizes (0.3–0.5 mm)	Collagen	Temporary—short duration	Lasts 4–7 days.	Lacrimedics

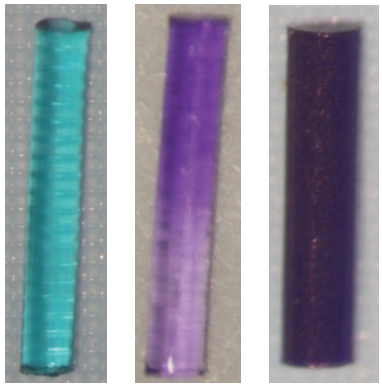


(a) (b)

Figure 6: Hydrogel vertical canalicular plug (Form Fit by Oasis). Dry (a) compared to wet (b) demonstrates that the plug becomes more gelatinous and enlarges slightly following hydration (10 minutes after application of 0.2 mL of water). Each dash in scale represents 1 mm.

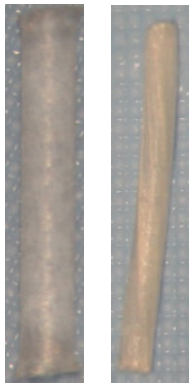
in aqueous tear volume and improved Schirmer I results, tear breakup time, and rose bengal staining [37]. Some studies have evaluated the SmartPlug in dry eye disease with a significant improvement in subjective symptoms and a decreased need for lubricants [38–40]. Kojima et al. reported no complications at 3-month follow-up after insertion of SmartPlug [39]. Although Schirmer test values were not significantly different before and after SmartPlug insertion, there was an improvement in rose bengal staining and a decrease in tear clearance rate. There is a possibility that these plugs do not fully occlude the canalicular lumen leading to an unchanged Schirmer test after plug insertion [39].

(ii) Refractive Surgery. Transient dry eye has been reported after laser surgery with a 59% incidence reported in a study 1 month after laser in situ keratomileusis (LASIK). Lacrimal plugs have a role in postrefractive surgery dry eyes and have been used preoperatively to prevent dry eye [41]. There are some controversies associated with the use of punctal and canalicular plugs in these scenarios. Occlusion can decrease the production of tears and reduce their clearance, which acts to worsen the dryness by increasing proinflammatory cytokines [42]. Yung et al. evaluated efficacy of punctal plugs in patients with post-LASIK dry eye [43]. The EaglePlug (EagleVision), a permanent silicone plug, was inserted a month after refractive surgery. Corneal sensitivity, Schirmer testing, and tear breakup time all improved in the treated group compared to the nontreatment group [43]. Albietz et al. reported that the use of lubricants and other options such as punctal plugs before refractive surgery increased



(a) (b) (c)

Figure 7: Comparison of extended duration plugs. (a) Soft Plug Extended Duration (Oasis)—extended duration temporary (3 months) plug made with a copolymer of glycolic acid and trimethylene carbonate. (b) Vera90 (Lacrivera)—extended duration temporary (3 months) plug made of E-Caprolactone-L-Lactide (PCL) copolymer. (c) VisiPlug (Lacrimedics)—extended duration temporary (6 months) plug made of polydioxanone (PDS).



(a) (b)

Figure 8: Comparison of temporary short duration plugs. (a) Lacrivera's Vera C7 made of collagen and effective for 7–10 days. (b) Oasis Soft Plug Collagen which is effective for 2–5 days.

the postprocedure goblet cell density [44]. A prospective randomized clinical trial evaluated punctal occlusion with punctal plugs after LASIK treatment for prevention of dry eye in 78 eyes of 39 patients [33]. Both eyes of the subjects underwent LASIK and lower punctal occlusion of one eye was performed while the other eye served as a control. At all follow-up visits the ocular surface index score was better and statistically significant for eyes with punctal plugs compared to the control eyes. At the 6-month final follow-up, although there was no statistically significant difference between the two eyes, the Schirmer I test, tear breakup time, and punctate epithelial keratitis scores were higher in the punctal plug occluded eyes than the control eyes. Kojima et al. evaluated preoperative insertion of punctal plugs to see its effects on postoperative vision and wound healing after laser epithelial keratomileusis [45]. Plugs were inserted both into the superior and inferior puncta. Significant improvement in the mean fluorescein score and mean uncorrected distance visual acuity was seen in the plug group compared to the nonplug group. Postoperative haze was less severe in the plug group. In another study, patients with low refractive errors (after refractive surgery) noted improvement in their visual acuity after silicone punctal plug placement [46]. Eighty-six percent of patients (7 eyes) gained at least one line of Snellen uncorrected visual acuity after punctal plug placement and decreased the desire to pursue further refractive surgery in 92% of the study group subjects. Collagen canalicular plugs can last from 3 days to 2 weeks and can improve symptoms of dry eye after laser refractive surgery by reducing flow through the canaliculus by 60–80% [47]. A literature review on prevention and treatment of LASIK-associated dry eye recommends treatment with artificial tears, punctal occlusion, topical cyclosporine A, and nutritional supplements prior to LASIK. It decreases the incidence of troublesome symptoms following laser surgery [48]. Huang at al. reported improvement in goblet cell density, corneal wound healing, and visual acuity in patients with temporary punctal occlusion after laser refractive surgery [49].

(iii) Contact Lens Wearers. Contact lens wearers with symptoms of dry eyes can benefit from punctal and canalicular plugs. Increased tear retention improves the symptoms of dry eye. Li et al. used ultrahigh resolution optical coherence tomography to see the effect of punctal occlusion on tear menisci in contact lens wearers with and without symptoms of dry eyes. Tear menisci increased transiently in both symptomatic and asymptomatic lens wearers and increased for a longer duration in symptomatic wearers [50]. A randomized controlled clinical trial evaluated the effect of punctal occlusion in dry eye contact lens wearers using a self-assessment questionnaire and evaluation of pre- and postlens tear film thickness. Extended wear intracanalicular plugs were used. Both the plug group and the sham group had significant improvement in their symptom scores. The effect of punctal occlusion did not differ between the two groups in terms of questionnaire score and treatment benefit assessment. It may indicate that punctal occlusion has no beneficial effect or the treatment effect was not detected due to a small sample size, nonparametric testing, or spontaneous plug extrusion [51]. Virtanen and colleagues observed a short lasting subjective and objective improvement in signs and symptoms after placement of horizontal canalicular plugs in contact lens wearers with both tear film deficiency and lens intolerance [52]. Intracanalicular plugs cannot be visualized directly making it difficult to exclude the possibility of migration or extrusion during the follow-up period. Improvement of symptoms and signs of dry eye disease was seen with insertion of silicone punctal plugs in a contact lens wearer with Sjogren's syndrome [53]. Silicone punctal plugs were inserted monocularly in lower puncta of 25 contact lens wearers with symptoms of dry eye [54]. Eighteen of the twenty-five patients reported a 34.6% increase in comfortable contact lens wear time at the 3-week follow-up.

(iv) Topical Medication Retention. Sustained delivery of ocular medications in patients with glaucoma and dry eye disease is needed and punctal plugs can reduce the dose of the drugs by attaining effective drug concentration while minimizing the risk of side effects. Punctal plugs used for drug delivery are made from various polymers and composed of an optional cap containing pores, optional outer shell that is impermeable to the drug and tears, cylindrical body containing the drug compound, and an optional unit for retaining the plug over a long period of time. The cap can have one or more pores for the release of drugs and can extend throughout the body. The head portion rests on the exterior of the punctum and the bottom end is tapered or narrower for easy insertion [55]. Recently canalicular plugs made from thermosensitive, hydrophobic, acrylic material (SmartPlug) have been used for ocular drug delivery with better retention [56]. The latanoprost punctal plug delivery system has been recently used for treatment of primary open-angle glaucoma and ocular hypertension. It has completed a phase II clinical trial and has shown promising results [57]. An olopatadine punctal plug drug delivery system has been used in patients with allergic conjunctivitis but has not shown significant efficacy compared to placebo delivery system [58]. There are reports on cyclosporine and moxifloxacin releasing punctal plug models being developed and used for delivery of these drugs [59, 60]. This approach can improve the quality of life for many patients.

(v) Acquired Punctal Stenosis. Konuk and colleagues evaluated perforated punctal plugs coated in PVP (polyvinylpyrrolidone) to treat complete and partial punctal stenosis in 44 eyes. The plugs were removed after 2 months with a mean follow-up period of 19 months. Success was achieved in 84.1% of eyes with relief of epiphora although a few cases had recurrence and mild horizontal lid laxity [27]. Chang et al. had similar results with a follow-up of more than 6 months [61]. Epiphora resolved in 85% patients. The patients with failure were all older than the success group and had associated chronic blepharitis. Wound healing occurring around the perforated punctal plug prevents restenosis. More prospective studies with larger sample sizes and longer follow-ups are needed to assess the effectiveness of perforated punctal plugs in treating partial and complete punctal occlusion. Punctal stenosis has also been treated with one-snip canaliculotomy and insertion of temporary punctal plugs to prevent restenosis [62].

(vi) Superior Limbic Keratoconjunctivitis. It has been observed that localized tear deficiency can cause friction between the upper lid and superior limbus resulting in symptoms of superior limbic keratoconjunctivitis (SLK). Upper punctal occlusion was used for management of refractory SLK and excellent results were obtained in all 22 eyes [63]. In one case report, administration of hydroxypropyl cellulose inserts improved symptoms of dry eye while SLK persisted in a patient with both Sjogren's syndrome and SLK. After several years of contact lens use the patient's symptoms reappeared and silicone punctal plugs were inserted, which improved both their dry eye disease and their superior limbic keratoconjunctivitis [53]. In another study SLK was an indication for placement of punctal silicone plugs in 11 eyes or 5.4% of the study group [64].

(vii) Postkeratoplasty Astigmatism. Collagen plugs have been implanted in radial keratotomy incisions to treat astigmatism after penetrating keratoplasty and eight of the eleven plugs were present several years later without any complications [65]. Espaillat et al. evaluated EagleVision collagen implants and treated high residual astigmatism after penetrating keratoplasty in 8 patients. Collagen plugs can be implanted as spacers between the relaxing incisions creating corneal flattening along the steep meridian. Although collagen implants usually do not last for more than a few days, Espaillat et al. observed that the implants can last up to 6 months in these grafts [66]. Collagen implants have been inserted in two live animal models with astigmatic keratotomy incisions and have been found to be safe and can enhance the effect of the incisions [67].

(viii) Others. Recurrent corneal erosions, epitheliopathy after penetrating keratoplasty, and persistent epithelial defects can also be managed with punctal and intracanalicular plugs. Tai et al. in a retrospective study observed that dry eye was the most common indication for silicone punctal plug insertion followed by epitheliopathy after penetrating keratoplasty (15.8%) [64]. Intraepithelial erosions during LASIK can be managed with punctal plugs, autologous serum drops, topical antibiotics, and bandage contact lenses. A female patient with a history of kidney disease developed recurrent epithelial erosions after LASIK and was managed with topical medications, soft bandage contact lens, and insertion of punctal plugs [68].

The indications of punctal and canalicular plugs are summarized in as follows.

Indications of Punctal and Canalicular Plugs

(i) Dry eye disease.
(ii) Contact lens wearers.
(iii) Punctal stenosis.
(iv) Refractive surgery.
(v) Post keratoplasty.
(vi) Topical medication delivery.
(vii) Superior limbic keratoconjuctivitis.
(viii) Recurrent corneal erosions.

2.5. Complications of Punctal and Intracanalicular Plugs

2.5.1. Punctal Plugs

(i) Extrusion, Granulation, Migration, and Enlargement of Punctal Size. Extrusion has been commonly reported with silicone punctal plugs occurring at a rate of 25–50% reported over the course of a month to 2 years after placement of these devices [32, 64, 69]. Sonomura et al. investigated complications with the SuperEagle Plug (EagleVision). The

study involved 148 puncta of 64 eyes. The extrusion rate was 57.4% in the follow-up period with no change in the size of the puncta or migration. Granulation was seen in 34.5% of patients [70]. A similar study done in Japan compared the EaglePlug, PunctalPlug, EagleFlex, and SuperFlex plugs to evaluate migration, extrusion, and enlargement of punctal size after extrusion in 291 eyes. They found that the time to extrusion was longer for SuperFlex plug than for others. Granulation tissue formed in 1.7% of the SuperFlex cases. In all the cases, a significant enlargement in the size of punctum was seen after extrusion [71]. Complete plug extrusion has the risk of enlarging the puncta, making reextrusion likely. A study on FCI punctal plugs with a slanted collarette was conducted with an observation period of 8 years. Retention rate was 84.2% after three months and decreased to 55.8% after a median of 2 years. Canalicular stenosis was seen after extrusion in 34.2% cases after 2 years. FCI plugs are harder than EaglePlugs (which are easier to insert and easy to remove) making retention better. It is also thought that the shape of FCI plug with the collarette with better fit lessens the foreign body sensation, minimizing the chances of extrusion [72]. Kaido et al. compared FCI silicone plugs with SuperFlex plugs (EagleVision) in a prospective interventional study [73]. The purpose was to investigate the retention rate and complications in relationship to the punctal size. Retention rate was 70.4% in the FCI plug F group compared to 30.1% with the SuperFlex at the 6-month follow-up. Spontaneous plug loss was attributed to a larger punctal size in patients with FCI plugs while old age with lid laxity was thought to be a contributory factor in patients with the SuperFlex. Punctal plug F is meant for insertion into puncta less than 0.8 mm in size. The high incidence of punctal plug extrusion has led to evolvement of new techniques to minimize the chances of this complication. Obata et al. described a technique to prevent reextrusion of punctal plugs. FlexPlugs of the same size as lost were sutured with 10-0 nylon in 10 puncta and 80% plugs were retained at 6 months [74]. To eliminate chances of plug migration, Kaido et al. used a plug size one diameter bigger than the measured punctal size. They inserted SuperFlex plugs and Soft plugs. No migration was seen at the 3-month follow-up period as compared to 13.8% with the standard technique [75]. In situations with severe dry eye and recurrent punctal plug extrusion, thermal cauterization is an effective treatment option with a very low recanalization rate [76]. Tai et al. reported a 49.4% retention rate of silicone punctal plugs with a mean survival time of 85.1 ± 7.3 weeks [64]. Most of the implants were lost within four weeks. Balaram et al. reported a 53% retention rate of punctal plugs after 6 months with a greater risk of extrusion in plugs placed in the upper versus lower puncta [32].

(ii) Pyogenic Granuloma. Pyogenic granuloma have been reported with both punctal and canalicular plugs. There is a case report of bilateral pyogenic granuloma with partial extrusion of perforated plugs in a patient 2 months after placement of the plug [77]. Musadiq et al. reported 2 cases of pyogenic granuloma occurring 3 months after insertion of Soft plugs [78]. Kim et al. in a retrospective observational case series with 903 silicone plugs (Parasol Punctal Occluders) observed pyogenic granuloma leading to extrusion of plugs in 4.2% of all the plugs placed. They proposed that formation of pyogenic granuloma could be due to irregular surfaces of silicone punctal plugs or the nose of plugs damaging the canalicular mucosa [79]. Pyogenic granuloma can develop anywhere in the body in response to injury or chronic irritation and silicone punctal plugs can cause this type of injury. An ampullary pyogenic granuloma overlying the superior punctum was reported in a female patient 14 months after placement of a silicone punctal plug. Both the plug and the granuloma were removed and a new silicone plug was inserted without complication [80].

(iii) Punctal and Canalicular Stenosis. Punctal plugs are used for reversible punctal occlusion and can cause punctal scarring and canalicular stenosis after extrusion or spontaneous loss. SuperEagle has been associated with canalicular stenosis in 34.2% of cases at 2 years [70]. Boldin et al. evaluated 17 eyes that developed punctal and canalicular stenosis after the loss of FCI punctal plugs and followed them up for a year [81]. The exact cause of stenosis was not known. It was thought to be attributed to the slanted collarette shape of the FCI plug damaging the punctal mucosa. This plug's unique shape necessitates rotation for a best fit. Other reasons postulated include collection of debris around the plug leading to chronic inflammation and scarring. It is thought that extrusion might be secondary to stenosis rather than stenosis secondary to extrusion. To determine the exact cause, larger studies need to be conducted and compare different punctal plugs to find any association of shape and designs with stenosis.

(iv) Canaliculitis and Dacryocystitis. A study has reported 2 cases of spontaneous migration of EagleVision tapered shaft punctal plugs into the canalicular system causing canaliculitis and dacryocystitis [82]. Although chances of migration of punctal silicone plugs are less than canalicular plugs, it can still occur. Newer smaller sized plugs are more prone to distal migration and can lead to infection. Eye rubbing and a dilated punctum can be additional contributory factors. Another case of canaliculitis 30 months after punctal occlusion was reported in Japan [83]. Two cases of *Aspergillus fumigatus* infection with SuperFlex (EagleVision) and FCI silicone plugs were reported [84]. The exact cause of fungal infection was not known, but the possibility of the plug insertion being related could not be excluded.

(v) Epiphora. Permanent punctal plugs have been associated with epiphora [85]. Epiphora has been reported in 10% of patients with punctal plugs in a report by the American Academy of Ophthalmology [30]. Another study reported epiphora in 11 eyes (5.4%) after insertion of silicone punctal plugs [64]. Shi et al. reported epiphora in 4 eyes (6.15%) [86].

(vi) Biofilm Formation. Punctal silicone plugs due to their exposed position and their complex shape can be easily contaminated with microbes resulting in an infection. In more than 50% of cases, *Staphylococcus* has been isolated from the culture of these contaminated plugs. There is some

evidence that acrylic plugs may portend a lower risk of infection than silicone plugs [9]. It has been observed that the hole of punctal plugs can be associated with bacterial biofilm. Sugita et al. evaluated Ready-Set FCI punctal plugs with scanning electron microscopy and cultured material extracted from plugs for presence of bacteria in 21 patients with severe dry eye disease. Positive cultures were seen in 44% of the sample material extracted from the plugs. *Staphylococcus epidermidis* was the commonest organism isolated (75%) followed by *Staphylococcus aureus* (25%) [87]. It is very important to carefully monitor these plugs for any accumulation of material or related signs in order to prevent future infections.

(vii) Discomfort. Localized discomfort has been associated with punctal plugs and some studies have reported this complication. Horwath-Winter et al. described localized discomfort with FCI silicone plugs in 2% of patients 34 months after placement of these plugs [72]. Balaram et al. reported localized discomfort with the EagleVision tapered shaft plug and the Oasis Soft Plug that was judged immediately and 3 months after plug placement [32]. Sugita et al. inserted silicone punctal plugs in 65 eyes and the most frequent complication observed was foreign body sensation [87].

(viii) Punctal Plug Surface Defects. The Quintess silicone punctal occluder with reservoir indentations was found to have punctal plug surface defects in 3 patients with local irritation of conjunctiva and inferonasal cornea [88]. These findings were observed 9, 40, and 69 months after their placement for symptoms of dry eye. These plugs were removed and found to have defect in collarette on scanning electron microscopy with sharp edges on the periphery. The irregular surface was also observed in the unused plugs under higher magnification.

2.5.2. Intracanalicular Plugs

(i) Allergic Reaction. Collagen absorbable plugs are made of bovine collagen, which is generally well tolerated. However, approximately 3% of the population is allergic to bovine collagen. Some studies have reported a granulomatous foreign body reaction with bovine collagen [89]. Ahn et al. reported a case of canaliculitis and a papilloma-like mass, three years after insertion of the plug [15]. Although collagen plugs usually dissolve in a few days, the possibility of retention cannot be excluded which mediates longer follow-up.

(ii) Canaliculitis, Dacryocystitis, and Other Infections. Intracanalicular plugs are placed in the horizontal or vertical canaliculus and are made of different materials. The SmartPlug is made of thermosensitive acrylic material and has many advantages including minimal chance of foreign body sensation, corneal erosion, or extrusion given its intracanalicular location. Its removal is usually easy to achieve with lacrimal irrigation. A SmartPlug study group reviewed 28 patient charts with SmartPlug insertion and complications treated by ophthalmic and plastic reconstructive surgeons [90]. Of these 28 patients, 64.3% developed complications including canaliculitis, dacryocystitis, and conjunctivitis. Patients were managed differently depending on the severity of complications. Intracanalicular position can increase the chance of infection making removal of the plug necessary. Hill et al. reported the prevalence of canaliculitis to be 4.73% per SmartPlug inserted. The average time to develop symptoms after insertion was 3 years. The patients were treated with canaliculotomy and plug removal [91]. Plug removal by irrigation failed in all cases; thus surgical intervention was necessary for every eye. The control group with punctal plugs had a lower complication rate of 2.1% at 2-year follow-up. Gerding et al. reported bilateral canaliculitis in a patient 2 years after placement of Herrick plugs. Surgical intervention and resection of cicatrized canaliculi were performed [92]. Lacrimal irrigation is considered an option for plug removal, but it is not always effective and can cause more inflammation resulting in scarring and worsening of the infection. Hill et al. suggested canaliculotomy for removal of these plugs, but this procedure has its own complications. M. Zhang and X. Zhang recently suggested a new method for removal of SmartPlugs. They used a lid clamp to flip the lid outward and if the size of the puncta was large enough no tools were needed for removal of the plug. If the size of the puncta was small, micro forceps were used for punctal dilation before application of the lid clamp, making removal easy [28]. Mazow et al. have reported canaliculitis occurring more frequently with intracanalicular plugs than the punctal ones [93]. Theoretically, canalicular position makes the removal of the plugs easier by irrigation, but this may not be the case as the plug can get lodged in the lower canalicular system and increase the chances of complications. Sixty-six (6.9%) out of nine hundred ninety-eight surgical cases developed complications (60 Herrick plugs, 6 SmartPlugs) requiring removal of the plugs. Five eyes developed canaliculitis and 29 eyes developed dacryocystitis and needed surgical treatment. Rabensteiner et al. compared SmartPlugs with silicone punctal plugs in the treatment of dry eye with a follow-up period of 3 months and found no significant difference between the two groups [26]. They reported that the SmartPlug does not fully occlude the canalicular lumen and, thus, allows tears to pass through. Chen and Lee reported significant improvement in dry eye symptoms in 91 eyes of 54 patients after SmartPlug insertion, but canaliculitis was reported in 6 eyes [38]. A survey was undertaken involving the American Society of Ophthalmic and Plastic and Reconstructive Surgery (ASOPRS) members' experiences with Herrick plugs. Among the 61% respondents that reported complications after plug placement, only 25% reported successful plug removal with lacrimal irrigation. Cases have been reported where a patient had multiple silicone intracanalicular plugs placed in the past and developed *Nocardia* canaliculitis, dacryocystitis, and subperiosteal abscess. A second patient developed dacryocystitis needing surgery [94]. Complications have also been associated with Form Fit plugs placed in the vertical canaliculus. Joganathan et al. reported 3 cases with complications of *Klebsiella* canaliculitis, canalicular abscess, and granulation tissue [95]. Ultrasound biomicroscopy can

Table 5: Complications of punctal and intracanalicular plugs.

Type of plug	Complications
Punctal plugs	(i) Extrusion (most common)
	(ii) Granulation tissue
	(iii) Enlargement of punctal size
	(iv) Migration (less common than canalicular plugs)
	(v) Canalicular stenosis
	(vi) Foreign body sensation
	(vii) Pyogenic granuloma
	(viii) Canaliculitis
	(ix) Dacryocystitis
	(x) Fungal/bacterial infections
	(xi) Epiphora
	(xii) Corneal ulceration
Canalicular plugs	(i) Allergy
	(ii) Granulomatous foreign body reaction
	(iii) Canaliculitis and dacryocystitis (more common than punctal plugs)
	(iv) Difficult removal
	(v) Klebsiella canaliculitis
	(vi) Pyogenic granuloma
	(vii) Epiphora
	(viii) Migration
	(ix) Canalicular stenosis

be used as an efficient diagnostic tool to visualize position of a retained intracanalicular plug [96].

(iii) Pyogenic Granuloma. There is a case report of pyogenic granuloma developing 2 years after insertion of the SmartPlug [97]. In a similar case report, a 65-year-old female patient developed a pyogenic granuloma in her left eye three years after insertion of bilateral SmartPlugs. Two weeks later a new granuloma appeared and both the plug and granuloma were removed [98]. A 47-year-old female patient developed ampullary pyogenic granuloma over the left superior punctum after insertion of silicone lacrimal plug. The plug had migrated to the common canaliculus and had to be removed surgically [99]. A retrospective study evaluated 66 eyes with complications after placement of Herrick plugs and SmartPlugs; pyogenic granulomas were observed in 11% of the eyes [93].

(iv) Epiphora. White et al. reported complications related to Herrick plugs in 41 patients who had symptomatic epiphora after plug insertion. Simple irrigation was not able to remove the plug and in most cases dacryocystorhinostomy was performed [100]. One theoretical advantage of Herrick plugs is easy removal by lacrimal irrigation; however this can be difficult leading to permanent obstruction of the lacrimal drainage system. Jones et al. observed that 10% of patients with Herrick plugs underwent an adverse event; epiphora was the most common followed by plug migration. Epiphora resolved with plug removal with saline flush in all but three patients [101]. Epiphora requiring plug removal was reported in 5.5% eyes after SmartPlug insertion [38]. Epiphora has been reported with canalicular plugs in another retrospective study [93]. Complications of punctal and intracanalicular plugs are summarized in Table 5.

(v) Plug Extrusion and Distal Migration. Due to the intracanalicular position of these plugs, there is a lower risk of extrusion compared to punctal plugs. Chen and Lee evaluated SmartPlugs in 91 eyes and reported spontaneous plug loss in 2 eyes [38]. Distal migration has been associated with canalicular plugs. Soparkar et al. reported distal migration of permanent lacrimal plugs in 12 patients causing symptoms that warranted removal [102]. Mazow et al. had reported lodged intracanalicular plugs causing lacrimal obstruction in 66 eyes [93].

2.6. Contraindications of Punctal and Intracanalicular Plugs. The use of these lacrimal occlusive devices is contraindicated in patients who are allergic to any of the materials as well as in patients with lacrimal outflow obstruction, ectropion, and active ocular infection [30]. Infectious conjunctivitis, in particular, is a contraindication to the use of punctal plugs [64]. Severe inflammatory changes of the ocular surface and the lids (such as blepharitis) should be treated prior to insertion of punctal plugs to reduce proinflammatory cytokines that can exacerbate inflammation [9].

3. Conclusion

A wide variety of punctal and canalicular plugs are available in the market. Their use is not only limited to nonpharmacological management of dry eyes but is gaining popularity in several other ophthalmic diseases. Newer designs are being made to decrease the risk of complications. Nevertheless there are limitations of these plugs and close monitoring is needed after placement. Future studies are needed comparing different types of plugs and following outcomes over longer timeframes. With new technology and ongoing research punctal plugs will continue to have an important role in the management of a myriad of eye conditions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] P. D. O'Brien and L. M. T. Collum, "Dry eye: diagnosis and current treatment strategies," *Current Allergy and Asthma Reports*, vol. 4, no. 4, pp. 314–319, 2004.

[2] N. A. Listed, "The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[3] A. S. Janine, "The epidemiology of dry eye disease: report of the epidemiology subcommittee of the International Dry Eye Workshop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 93–107, 2007.

[4] D. A. Schaumberg, D. A. Sullivan, J. E. Buring, and M. R. Dana, "Prevalence of dry eye syndrome among US women," *American Journal of Ophthalmology*, vol. 136, no. 2, pp. 318–326, 2003.

[5] D. A. Schaumberg, R. Dana, J. E. Buring, and D. A. Sullivan, "Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies," *Archives of Ophthalmology*, vol. 127, no. 6, pp. 763–768, 2009.

[6] J. L. Gayton, "Etiology, prevalence, and treatment of dry eye disease," *Clinical Ophthalmology*, vol. 3, no. 1, pp. 405–412, 2009.

[7] A. Behrens, J. J. Doyle, L. Stern et al., "Dysfunctional tear syndrome: a Delphi approach to treatment recommendations," *Cornea*, vol. 25, no. 8, pp. 900–907, 2006.

[8] S. C. Pflugfelder, G. Geerling, S. Kinoshita et al., "Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop," *The Ocular Surface*, vol. 5, no. 2, pp. 163–178, 2007.

[9] F. Tost and G. Geerling, "Plugs for occlusion of the lacrimal drainage system," in *Surgery for the Dry Eye*, G. Geerling and H. Brewitt, Eds., vol. 41 of *Developments in Ophthalmology*, pp. 193–212, Karger Publishers, Basel, Switzerland, 2008.

[10] A. Lemp, "Report of the National Eye Institute/industry workshop on clinical trials in dry eyes," *The CLAO Journal*, vol. 21, no. 4, pp. 221–232, 1995.

[11] B. Lipková, "Perforated punctum plugs in treatment lacrimal punctal stenosis," *Česká a Slovenská Oftalmologie*, vol. 68, no. 4, pp. 147–149, 2012.

[12] C. W. Roberts, P. E. Carniglia, and B. G. Brazzo, "Comparison of topical cyclosporine, punctal occlusion, and a combination for the treatment of dry eye," *Cornea*, vol. 26, no. 7, pp. 805–809, 2007.

[13] L. J. Maguire and G. B. Bartley, "Complications associated with the new smaller size freeman punctal plug," *Archives of Ophthalmology*, vol. 107, no. 7, pp. 961–962, 1989.

[14] S. A. Baxter and P. R. Laibson, "Punctal plugs in the management of dry eyes," *The Ocular Surface*, vol. 2, no. 4, pp. 255–265, 2004.

[15] H. B. Ahn, J. W. Seo, M. S. Roh, W. J. Jeong, W. C. Park, and S. H. Rho, "Canaliculitis with a papilloma-like mass caused by a temporary punctal plug," *Ophthalmic Plastic & Reconstructive Surgery*, vol. 25, no. 5, pp. 413–414, 2009.

[16] T. C. Huang and D. A. Lee, "Punctal occlusion and topical medications for glaucoma," *American Journal of Ophthalmology*, vol. 107, no. 2, pp. 151–155, 1989.

[17] V. K. Yellepeddi, R. Sheshala, H. McMillan, C. Gujral, D. Jones, and T. Raghu Raj Singh, "Punctal plug: a medical device to treat dry eye syndrome and for sustained drug delivery to the eye," *Drug Discovery Today*, vol. 20, no. 7, pp. 884–889, 2015.

[18] 2015, http://www.beaver-visitec.com/, http://www.odysseymed.com/.

[19] 2015, http://www.oasismedical.com/.

[20] Eagle Vision, September 2015, http://www.dryeye.org.

[21] 2015, http://www.lacrivera.com/.

[22] Lacrimedics, September 2015, http://www.lacrimedics.com.

[23] 2015, http://www.medennium.com/.

[24] 2015, http://www.surgicalspecialties.com.

[25] 2015, http://www.alphamedco.com.

[26] D. F. Rabensteiner, I. Boldin, A. Klein, and J. Horwath-Winter, "Collared silicone punctal plugs compared to intracanalicular plugs for the treatment of dry eye," *Current Eye Research*, vol. 38, no. 5, pp. 521–525, 2013.

[27] O. Konuk, B. Urgancioglu, and M. Unal, "Long-term success rate of perforated punctal plugs in the management of acquired punctal stenosis," *Ophthalmic Plastic & Reconstructive Surgery*, vol. 24, no. 5, pp. 399–402, 2008.

[28] M. Zhang and X. Zhang, "New method for removing thermosensitive acrylic punctal plugs from lacrimal puncta," *Cornea*, vol. 34, no. 12, pp. 1557–1559, 2015.

[29] M. Taban, B. Chen, and J. D. Perry, "Update on punctal plugs," *Comprehensive Ophthalmology Update*, vol. 7, no. 5, pp. 205–212, 2006.

[30] M. M. Marcet, R. M. Shtein, E. A. Bradley et al., "Safety and efficacy of lacrimal drainage system plugs for dry eye syndrome: a report by the American Academy of Ophthalmology," *Ophthalmology*, vol. 122, no. 8, pp. 1681–1687, 2015.

[31] A. Sy, K. S. O'Brien, M. P. Liu et al., "Expert opinion in the management of aqueous Deficient Dry Eye Disease (DED)," *BMC Ophthalmology*, vol. 15, article 133, 2015.

[32] M. Balaram, D. A. Schaumberg, and M. R. Dana, "Efficacy and tolerability outcomes after punctal occlusion with silicone plugs in dry eye syndrome," *American Journal of Ophthalmology*, vol. 131, no. 1, pp. 30–36, 2001.

[33] A. M. Alfawaz, S. Algehedan, S. S. Jastaneiah, S. Al-Mansouri, A. Mousa, and A. Al-Assiri, "Efficacy of punctal occlusion in management of dry eyes after laser in situ keratomileusis for myopia," *Current Eye Research*, vol. 39, no. 3, pp. 257–262, 2014.

[34] A. Nava-Castaneda, J. L. Tovilla-Canales, L. Rodriguez, J. L. Tovilla y Pomar, and C. E. Jones, "Effects of lacrimal occlusion with collagen and silicone plugs on patients with conjunctivitis associated with dry eye," *Cornea*, vol. 22, no. 1, pp. 10–14, 2003.

[35] J. P. Gilbard, S. R. Rossi, D. T. Azar, and K. G. Heyda, "Effect of punctal occlusion by Freeman silicone plug insertion on tear osmolarity in dry eye disorders," *The CLAO Journal*, vol. 15, no. 3, pp. 216–218, 1989.

[36] D. Dursun, A. Ertan, B. Bilezikçi, Y. A. Akova, and A. Pelit, "Ocular surface changes in keratoconjunctivitis sicca with silicone punctum plug occlusion," *Current Eye Research*, vol. 26, no. 5, pp. 263–269, 2003.

[37] R. Altan-Yaycioglu, E. A. Gencoglu, Y. A. Akova, D. Dursun, F. Cengiz, and A. Akman, "Silicone versus collagen plugs for treating dry eye: results of a prospective randomized trial including lacrimal scintigraphy," *American Journal of Ophthalmology*, vol. 140, no. 1, pp. 88.e1–88.e7, 2005.

[38] S. X. Chen and G. A. Lee, "SmartPlug in the management of severe dry eye syndrome," *Cornea*, vol. 26, no. 5, pp. 534–538, 2007.

[39] T. Kojima, M. Dogru, R. Ishida, E. Goto, Y. Matsumoto, and K. Tsubota, "Clinical evaluation of the Smart Plug™ in the treatment of dry eyes," *American Journal of Ophthalmology*, vol. 141, no. 2, pp. 386–388, 2006.

[40] L. Guo, X.-Y. Wu, F. Yuan, and B. Li, "Application of Smart Plug lacrimal plug in the treatment of dry eye," *Journal of Southern Medical University*, vol. 30, no. 10, pp. 2370–2372, 2010.

[41] E. Y. W. Yu, A. Leung, S. Rao, and D. S. C. Lam, "Effect of laser in situ keratomileusis on tear stability," *Ophthalmology*, vol. 107, no. 12, pp. 2131–2135, 2000.

[42] M. T. Yen, S. C. Pflugfelder, and W. J. Feuer, "The effect of punctal occlusion on tear production, tear clearance, and ocular surface sensation in normal subjects," *American Journal of Ophthalmology*, vol. 131, no. 3, pp. 314–323, 2001.

[43] Y. H. Yung, I. Toda, C. Sakai, A. Yoshida, and K. Tsubota, "Punctal plugs for treatment of post-LASIK dry eye," *Japanese Journal of Ophthalmology*, vol. 56, no. 3, pp. 208–213, 2012.

[44] J. M. Albietz, S. G. McLennan, and L. M. Lenton, "Ocular surface management of photorefractive keratectomy and laser in situ keratomileusis," *Journal of Refractive Surgery*, vol. 19, no. 6, pp. 636–644, 2003.

[45] T. Kojima, T. Watabe, T. Nakamura, K. Ichikawa, and Y. Satoh, "Effects of preoperative punctal plug treatment on visual function and wound healing in laser epithelial keratomileusis," *Journal of Refractive Surgery*, vol. 27, no. 12, pp. 894–898, 2011.

[46] M. B. Khalil, R. A. Latkany, M. G. Speaker, and G. Yu, "Effect of punctal plugs in patients with low refractive errors considering refractive surgery," *Journal of Refractive Surgery*, vol. 23, no. 5, pp. 467–471, 2007.

[47] R. T. Ang, D. A. Dartt, and K. Tsubota, "Dry eye after refractive surgery," *Current Opinion in Ophthalmology*, vol. 12, no. 4, pp. 318–322, 2001.

[48] R. Ambrósio Jr., T. Tervo, and S. E. Wilson, "LASIK-associated dry eye and neurotrophic epitheliopathy: pathophysiology and strategies for prevention and treatment," *Journal of Refractive Surgery*, vol. 24, no. 4, pp. 396–407, 2008.

[49] B. Huang, M. A. Mirza, M. A. Qazi, and J. S. Pepose, "The effect of punctal occlusion on wavefront aberrations in dry eye patients after laser in situ keratomileusis," *American Journal of Ophthalmology*, vol. 137, no. 1, pp. 52–61, 2004.

[50] M. Li, J. Wang, M. Shen et al., "Effect of punctal occlusion on tear menisci in symptomatic contact lens wearers," *Cornea*, vol. 31, no. 9, pp. 1014–1022, 2012.

[51] J. R. Geldis and J. J. Nichols, "The impact of punctal occlusion on soft contact lens wearing comfort and the tear film," *Eye & Contact Lens*, vol. 34, no. 5, pp. 261–265, 2008.

[52] T. Virtanen, K. Huotari, M. Härkönen, and T. Tervo, "Lacrimal plugs as a therapy for contact lens intolerance," *Eye*, vol. 10, no. 6, pp. 727–731, 1996.

[53] A. H. Wander, "Long-term use of hydroxypropyl cellulose ophthalmic insert to relieve symptoms of dry eye in a contact lens wearer: case-based experience," *Eye & Contact Lens*, vol. 37, no. 1, pp. 39–44, 2011.

[54] D. Giovagnoli and S. J. Graham, "Inferior punctal occlusion with removable silicone punctal plugs in the treatment of dry-eye related contact lens discomfort," *Journal of the American Optometric Association*, vol. 63, no. 7, pp. 481–485, 1992.

[55] U. B. Kompella, R. S. Kadam, and V. H. L. Lee, "Recent advances in ophthalmic drug delivery," *Therapeutic Delivery*, vol. 1, no. 3, pp. 435–456, 2010.

[56] N. Gooch, S. A. Molokhia, R. Condie et al., "Ocular drug delivery for glaucoma management," *Pharmaceutics*, vol. 4, no. 1, pp. 197–211, 2012.

[57] Press release, http://www.qltinc.com/newsCenter/2009/090728a1.htm.

[58] Press release, http://www.qltinc.com/newsCenter/2012/121025.htm.

[59] C. Gupta and A. Chauhan, "Ophthalmic delivery of cyclosporine A by punctal plugs," *Journal of Controlled Release*, vol. 150, no. 1, pp. 70–76, 2011.

[60] S.-P. Chee, "Moxifloxacin punctum plug for sustained drug delivery," *Journal of Ocular Pharmacology and Therapeutics*, vol. 28, no. 4, pp. 340–349, 2012.

[61] M. Chang, S.-E. Ahn, and S. Baek, "The effect of perforated punctal plugs in the management of acquired punctal stenosis," *Journal of Craniofacial Surgery*, vol. 24, no. 5, pp. 1628–1630, 2013.

[62] R. W. Kristan, "Treatment of lacrimal punctal stenosis with a one-snip canaliculotomy and temporary punctal plugs," *Archives of Ophthalmology*, vol. 106, no. 7, pp. 878–879, 1988.

[63] H.-Y. Yang, H. Fujishima, I. Toda, J. Shimazaki, and K. Tsubota, "Lacrimal punctal occlusion for the treatment of superior limbic keratoconjunctivitis," *American Journal of Ophthalmology*, vol. 124, no. 1, pp. 80–87, 1997.

[64] M.-C. Tai, C. B. Cosar, E. J. Cohen, C. J. Rapuano, and P. R. Laibson, "The clinical efficacy of silicone punctal plug therapy," *Cornea*, vol. 21, no. 2, pp. 135–139, 2002.

[65] D. Baskin and S. Waller, "Implantation of collagen punctal plugs for the reduction of post-keratoplasty ametropia," *Nepalese Journal of Ophthalmology*, vol. 1, no. 1, pp. 72–74, 2009.

[66] A. Espaillat, K. To, and E. M. Perlman, "Effect of collagen implants in the reduction of high astigmatism induced by penetrating keratoplasty," *Journal of Cataract & Refractive Surgery*, vol. 25, no. 10, pp. 1336–1340, 1999.

[67] S. G. Waller, K. LaMarche, and J. H. Talamo, "Implantation of collagen plugs into incisional keratotomies," *Journal of Cataract & Refractive Surgery*, vol. 22, no. 3, pp. 304–306, 1996.

[68] N. Asano-Kato, I. Toda, and K. Tsubota, "Severe late-onset recurrent epithelial erosion with diffuse lamellar keratitis after laser in situ keratomileusis," *Journal of Cataract & Refractive Surgery*, vol. 29, no. 10, pp. 2019–2021, 2003.

[69] A. Sakamoto, K. Kitagawa, and A. Tatami, "Efficacy and retention rate of two types of silicone punctal plugs in patients with and without Sjögren syndrome," *Cornea*, vol. 23, no. 3, pp. 249–254, 2004.

[70] Y. Sonomura, N. Yokoi, A. Komuro, K. Inagaki, and S. Kinoshita, "Clinical investigation of the extrusion rate and other complications of the SuperEagle plug," *Nippon Ganka Gakkai Zasshi*, vol. 117, no. 2, pp. 126–131, 2013.

[71] N. Nasu, N. Yokoi, M. Nishii, A. Komuro, K. Inagaki, and S. Kinoshita, "Clinical investigation of the extrusion rate and other complications of the new Super Flex Plug punctal plug and other plugs," *Nippon Ganka Gakkai Zasshi*, vol. 112, no. 7, pp. 601–606, 2008.

[72] J. Horwath-Winter, A. Thaci, A. Gruber, and I. Boldin, "Long-term retention rates and complications of silicone punctal plugs in dry eye," *American Journal of Ophthalmology*, vol. 144, no. 3, pp. 441.e1–444.e1, 2007.

[73] M. Kaido, R. Ishida, M. Dogru, and K. Tsubota, "Comparison of retention rates and complications of 2 different types of silicon lacrimal punctal plugs in the treatment of dry eye disease," *American Journal of Ophthalmology*, vol. 155, no. 4, pp. 648–653, 2013.

[74] H. Obata, N. Ibaraki, and T. Tsuru, "A technique for preventing spontaneous loss of lacrimal punctal plugs," *American Journal of Ophthalmology*, vol. 141, no. 3, pp. 567–569, 2006.

[75] M. Kaido, R. Ishida, M. Dogru, and K. Tsubota, "A new punctal plug insertion technique to prevent intracanalicular plug migration," *American Journal of Ophthalmology*, vol. 147, no. 1, pp. 178.e1–182.e1, 2009.

[76] S. Yaguchi, Y. Ogawa, M. Kamoi et al., "Surgical management of lacrimal punctal cauterization in chronic GVHD-related dry eye with recurrent punctal plug extrusion," *Bone Marrow Transplantation*, vol. 47, no. 11, pp. 1465–1469, 2012.

[77] O. H. Ababneh and M. M. Msallam, "Bilateral simultaneous pyogenic granuloma after perforated punctal plug insertion," *Ophthalmic Plastic & Reconstructive Surgery*, vol. 30, no. 5, pp. e113–e115, 2014.

[78] M. Musadiq, S. Mukherji, and S. Sandramouli, "Pyogenic granuloma following silicone punctal plugs: report of two cases," *Orbit*, vol. 24, no. 2, pp. 149–151, 2005.

[79] B. M. Kim, S. S. Osmanovic, and D. P. Edward, "Pyogenic granulomas after silicone punctal plugs: a clinical and histopathologic study," *American Journal of Ophthalmology*, vol. 139, no. 4, pp. 678–684, 2005.

[80] Y. A. Akova, B. Demirhan, S. Çakmakçi, and P. Aydin, "Pyogenic granuloma: a rare complication of silicone punctal plugs," *Ophthalmic Surgery and Lasers*, vol. 30, no. 7, pp. 584–585, 1999.

[81] I. Boldin, A. Klein, E.-M. Haller-Schober, and J. Horwath-Winter, "Long-term follow-up of punctal and proximal canalicular stenoses after silicone punctal plug treatment in dry eye patients," *American Journal of Ophthalmology*, vol. 146, no. 6, pp. 968–972, 2008.

[82] S. Rumelt, H. Remulla, and P. A. D. Rubin, "Silicone punctal plug migration resulting in dacryocystitis and canaliculitis," *Cornea*, vol. 16, no. 3, pp. 377–379, 1997.

[83] M. Takemura, N. Yokoi, Y. Nakamura, A. Komuro, J. Sugita, and S. Kinoshita, "Canaliculitis caused by *Actinomyces* in a case of dry eye with punctal plug occlusion," *Nippon Ganka Gakkai Zasshi*, vol. 106, no. 7, pp. 416–419, 2002.

[84] K. F. Tabbara, "*Aspergillus fumigatus* colonization of punctal plugs," *American Journal of Ophthalmology*, vol. 143, no. 1, pp. 180–181, 2007.

[85] H. J. Glatt, "Failure of collagen plugs to predict epiphora after permanent punctal occlusion," *Ophthalmic Surgery*, vol. 23, no. 4, pp. 292–293, 1992.

[86] S. Shi, W. Chen, X. Zhang, H.-X. Ma, and L. Sun, "Effects of silicone punctal plugs for tear deficiency dry eye patients," *Chinese Journal of Ophthalmology*, vol. 49, no. 2, pp. 151–154, 2013.

[87] J. Sugita, N. Yokoi, N. J. Fullwood et al., "The detection of bacteria and bacterial biofilms in punctal plug holes," *Cornea*, vol. 20, no. 4, pp. 362–365, 2001.

[88] S. C. Paparizos, D. P. Edward, and S. Osmanovic, "Plug surface defects as a late complication of silicone punctal plugs," *Cornea*, vol. 32, no. 9, pp. 1224–1226, 2013.

[89] C. Raulin, B. Greve, W. Hartschuh, and K. Soegding, "Exudative granulomatous reaction to hyaluronic acid (Hylaform$^{\text{Ÿ}}$)," *Contact Dermatitis*, vol. 43, no. 3, pp. 178–179, 2000.

[90] SmartPlug Study Group, "Management of complications after insertion of the SmartPlug punctal plug: a study of 28 patients," *Ophthalmology*, vol. 113, no. 10, pp. 1859–1862, 2006.

[91] R. H. Hill, S. W. Norton, and T. A. Bersani, "Prevalence of canaliculitis requiring removal of SmartPlugs," *Ophthalmic Plastic & Reconstructive Surgery*, vol. 25, no. 6, pp. 437–439, 2009.

[92] H. Gerding, J. Küppers, and H. Busse, "Symptomatic cicatrizial occlusion of canaliculi after insertion of Herrick lacrimal plugs," *American Journal of Ophthalmology*, vol. 136, no. 5, pp. 926–928, 2003.

[93] M. L. Mazow, T. McCall, and T. C. Prager, "Lodged intracanalicular plugs as a cause of lacrimal obstruction," *Ophthalmic Plastic & Reconstructive Surgery*, vol. 23, no. 2, pp. 138–142, 2007.

[94] J. Lee and J. C. Flanagan, "Complications associated with silicone intracanalicular plugs," *Ophthalmic Plastic & Reconstructive Surgery*, vol. 17, no. 6, pp. 465–469, 2001.

[95] V. Joganathan, P. Mehta, A. Murray, and O. M. Durrani, "Complications of intracanalicular plugs: a case series," *Orbit*, vol. 29, no. 5, pp. 271–273, 2010.

[96] J. J. Hurwitz, C. J. Pavlin, and E.-K. Rhemtulla, "Identification of retained intracanalicular plugs with ultrasound biomicroscopy," *Canadian Journal of Ophthalmology*, vol. 39, no. 5, pp. 533–537, 2004.

[97] T. Y. Chou, H. D. Perry, and E. D. Donnenfeld, "Pyogenic granuloma formation following placement of the Medennium SmartPLUG punctum plug," *Cornea*, vol. 25, no. 4, pp. 493–495, 2006.

[98] F. Pastor-Pascual, J. Aviñó-Martínez, E. España-Gregori, and P. Alcocer-Yuste, "Pyogenic granuloma following Smart Plug insertion," *Archivos de la Sociedad Espanola de Oftalmologia*, vol. 82, no. 10, pp. 653–656, 2007.

[99] H.-C. Hsu, "Ampullary pyogenic granuloma as a complication of lacrimal plug migration," *Chang Gung Medical Journal*, vol. 25, no. 6, pp. 415–418, 2002.

[100] W. L. White, G. B. Bartley, M. J. Hawes, J. V. Linberg, and D. B. Leventer, "Iatrogenic complications related to the use of Herrick lacrimal plugs," *Ophthalmology*, vol. 108, no. 10, pp. 1835–1837, 2001.

[101] C. E. Jones, M. Anklesaria, A. D. Gordon et al., "Retrospective safety study of the herrick lacrimal plug: a device used to occlude the lacrimal canaliculus," *The CLAO Journal*, vol. 28, no. 4, pp. 206–210, 2002.

[102] C. N. S. Soparkar, J. R. Patrinely, J. Hunts, J. V. Linberg, R. C. Kersten, and R. Anderson, "The perils of permanent punctal plugs," *American Journal of Ophthalmology*, vol. 123, no. 1, pp. 120–121, 1997.

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Review Article

Review: The Lacrimal Gland and Its Role in Dry Eye

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The human tear film is a 3-layered coating of the surface of the eye and a loss, or reduction, in any layer of this film may result in a syndrome of blurry vision and burning pain of the eyes known as dry eye. The lacrimal gland and accessory glands provide multiple components to the tear film, most notably the aqueous. Dysfunction of these glands results in the loss of aqueous and other products required in ocular surface maintenance and health resulting in dry eye and the potential for significant surface pathology. In this paper, we have reviewed products of the lacrimal gland, diseases known to affect the gland, and historical and emerging dry eye therapies targeting lacrimal gland dysfunction.

1. Introduction

The human tear film coats the anterior surface of the eye and is composed of three distinct layers: an inner mucin coating, a middle aqueous component, and a lipid overlay. Traditionally, the mucin layer was felt to be derived from goblet cells of the conjunctiva, the aqueous component from the lacrimal gland, and the lipid layer from the meibomian glands [1–3]. Recent advancements in proteomics have slightly altered this view of the tear film by identifying mucin as a product of the goblet cells but the lacrimal gland as well [4]. The 3-layered tear film inhibits ocular surface invasion by pathogens, provides an air-tissue interface for gas exchange, and supplies essential nutrients and metabolites to maintain a transparent and avascular cornea. The lacrimal gland contributes multiple components to the tear film and has been the center of much research including multiple products now under clinical trials. In the this paper we review the anatomy, physiology, and normal products of the lacrimal gland in regard to their role in dry eye diseases. We have also reviewed specific causes of lacrimal gland pathology such as aging, smoking, autoimmune diseases, and infections. Finally, the historical and emerging treatments for dry eye related to lacrimal gland dysfunction with an emphasis on surgical approaches are detailed within.

2. Anatomy, Physiology, Innervation, and Histology

A proper review of the anatomy of the lacrimal gland and accessory lacrimal tissues is important for understanding the pathophysiology of dry eye syndrome and secondary causes of dry eye.

2.1. Anatomy, Blood Supply, Innervation. Embryologically, the main lacrimal gland develops from an outpouching of the conjunctiva. The accessory lacrimal glands develop slightly later than the main lacrimal gland [5]. The main lacrimal gland is situated superotemporally in the orbit within the lacrimal fossa of the frontal bone. Grossly, the gland is a pinkish-gray structure composed of small lobules intermixed with connective tissue septations and lacks a true capsule (Figure 1). Its appearance may be mistaken for preaponeurotic fat. The gland is divided into two lobes, the orbital and palpebral lobes, by the lateral horn of the levator aponeurosis. Although divided, the division is incomplete due to a posterior wall of parenchyma between the lobes [5]. The gland is bound anteriorly by the orbital septum and the preaponeurotic fat pad, posteriorly by orbital fat, medially by the intermuscular membrane between the superior and

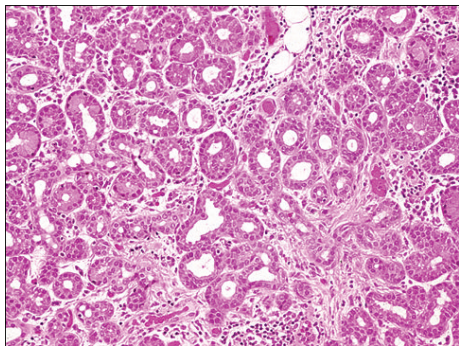

Figure 1: Lacrimal gland histopathology. H&E staining of a normal lacrimal gland. The gland is composed of lobules separated by loose connective tissue. The lobules are composed of multiple acini lined by columnar secretory cells.

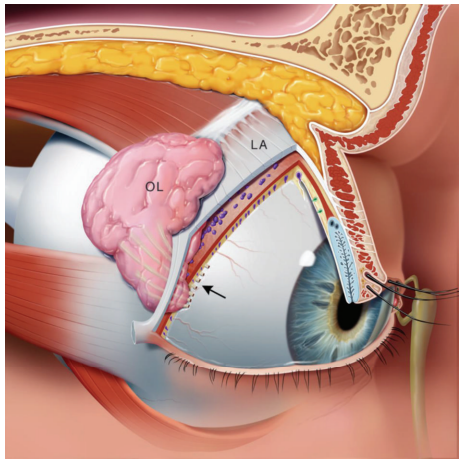

Figure 2: Oblique view of the right orbit. Oblique view of the right orbit showing the main lacrimal gland divided into the orbital lobe (OL) and palpebral lobe by the lateral horn of the levator aponeurosis (LA). Note the excretory ducts coursing through the palpebral lobe and draining into the superior conjunctival fornix (arrow).

lateral recti, and laterally by bone (Figure 2). The size of the main lacrimal gland is somewhat variable with the orbital lobe being the larger of the two. The gland averages approximately 20 mm long and 12 mm wide with the orbital and palpebral lobes having a thickness of 5 mm and 3 mm, respectively [6, 7]. The palpebral lobe lies beneath the levator aponeurosis in the subaponeurotic Jones' space [5]. The gland is supported by conjunctiva, intermuscular membranes, its facial attachments to Whitnall's ligament, and the levator horn (Figures 2 and 3).

The lacrimal gland is an exocrine gland similar to the mammary gland and salivary gland [7]. The gland is composed of lobules separated by loose connective tissue (Figure 1). Acini are lined with columnar secretory cells, which have been shown to secrete mucopolysaccharides, implying that the gland is a modified mucus gland [5]. Each lacrimal gland lobule consists of many acini and intralobular ducts that drain into approximately 8–12 excretory ducts

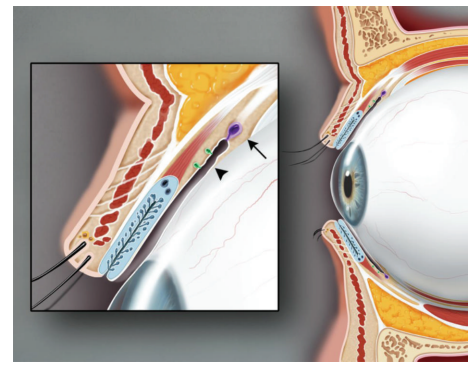

Figure 3: Sagittal view of the upper and lower eyelids. The glands of Krause (arrow) are located in the superior conjunctival fornix. The glands of Wolfring (arrowhead) are found at the nonmarginal border of the tarsal plate.

or tubules. The ducts of both the orbital and palpebral lobes drain into the superotemporal conjunctival fornix, approximately 5 mm superior the lateral tarsal border [8]. The ducts of the orbital lobe pass through the parenchyma of the palpebral lobe making the proximal secretory ducts susceptible to damage distally [5, 7, 8].

The arterial blood supply to the lacrimal gland comes from the lacrimal branch of the ophthalmic artery, a branch of the infraorbital artery, and occasionally from a branch of the recurrent meningeal artery. The lacrimal artery passes through the gland to feed the upper and lower eyelids. The lacrimal vein follows the course of the artery and drains into the superior ophthalmic vein.

The gland is innervated by both myelinated and unmyelinated fibers arising from the trigeminal nerve, the facial nerve, and sympathetic innervation from the superior cervical ganglion [5]. Stimulation of the ocular surface activates tear production from the main lacrimal gland (reflex tearing). The lacrimal nerve is a sensory branch of the ophthalmic trigeminal nerve (V_1), which provides the sensory (afferent) pathway. This lacrimal nerve travels in the superotemporal orbit and enters the gland with the major vessels. This nerve courses through the gland to innervate superficial eyelid structures. Sympathetic nerves travel with the lacrimal artery along with parasympathetics in the zygomatic nerve [5].

The efferent pathway originates with parasympathetic fibers from the superior salivary nucleus of the pons, which exit the brain stem with the facial nerve. Lacrimal fibers depart from the facial nerve as the greater superficial petrosal nerve and travel to the sphenopalatine ganglion to join the zygomatic nerve. The zygomatic nerve enters the orbit 5 mm posterior to the anterior limit of the inferior orbital fissure. Prior to dividing into the zygomaticotemporal and zygomaticofacial branches, the zygomatic nerve may give off a lacrimal branch, which may anastomose with a branch of the lacrimal nerve or travel independently along the periorbita [5]. It is unclear if the anastomosis between the zygomaticotemporal and lacrimal nerves is uniformly present [8]. The role of the sympathetic nervous system is thought to stimulate basal tear secretion, but its role in lacrimation is

not well understood. Lacrimal gland hyposecretion is seen in syndromes of central autonomic dysfunction, such as Riley-Day syndrome [9].

There are approximately 20 glands of Krause located in the superior conjunctival fornix and approximately half as many in the inferior fornix. The glands of Wolfring are found along the nonmarginal border of the both tarsal plates (Figures 2 and 3) [5]. Accessory lacrimal glands may also be found in the caruncle and in the plica semilunaris. The accessory glands account for approximately 10% of the total lacrimal secretory mass [8]. Although the accessory lacrimal glands of Krause and Wolfring are structurally and histologically similar to the main lacrimal gland and may develop identical types of metaplasia, they differ in their innervation [7]. Although heavily innervated, the accessory lacrimal glands lack parasympathetic innervation [5], and most of the innervation is unidentified [8]. Jones states that the main lacrimal gland is responsible only for reflex tearing and the accessory glands of Kraus and Wolfring, providing basal tear secretion [10]. This distinction has been debated. The volume of tears secreted from these glands is unclear. Studies show mixed results whether or not the accessory glands are able to provide adequate tear volume to prevent keratoconjunctivitis sicca [7].

2.2. Pathology. Noted age-related changes of the lacrimal gland include atrophy of the glandular parenchyma, increased interstitial connective tissue, increased fat content within glandular tissue and epithelial secretory cells, and increased lymphocyte content within the gland including plasma cells [11–13]. The incidence and uniformity of these changes have not been agreed upon, as many reports note conflicting data.

Obata et al. found that lobular fibrosis, lobular atrophy, diffuse fibrosis, diffuse atrophy, periductal fibrosis, lymphocytic foci, and fatty infiltration were found significantly more often in orbital lobes, whereas interlobular ductal dilatation was observed more frequently in palpebral lobes [11]. It is unknown if structural and functional differences exist between the orbital and palpebral lobes or if these differences represent continuum of changes versus distinct pathophysiologic changes.

An autopsy study of lacrimal glands by Roen and colleagues found that 75% of glands studied showed microscopic abnormalities [12]. The most common abnormal findings included chronic inflammation and periductular fibrosis. Approximately 52% and 74% of patients over the age of 50 showed signs of periductular fibrosis and ductal abnormalities, respectively [12]. The authors also observed massive ductular ectasia extending into lobules. The combination of periductular fibrosis, inflammation, and dilated, inspissated ducts may lead to retention of tears within the lacrimal gland and contribute to age-related dry eye [12]. Another study by Obata et al. found a statistically significant difference in incidence of diffuse fibrosis, atrophy, and periductal fibrosis of the lacrimal gland in postmenopausal women compared to men [11]. Glands in which acinar atrophy is apparent show a lack of lysozyme immunoreactivity and are probably related to a decrease of tear proteins as a consequence of aging [7]. Atrophy of acinar elements may result in fibrosis, but in certain conditions, such as chronic graft-versus-host disease, stromal fibroblasts are actively involved in the pathogenic process of periacinar fibrosis [14]. The health of the conjunctival epithelium is essential for normal lacrimal gland function. Stenosis or obstruction of flow of the excretory ducts in the superior conjunctival fornix may cause cystic dilatation of the interlobular ducts in the palpebral lobe. Damage to the excretory ducts in the superior conjunctiva may occur with severe ocular surface diseases with keratinization such as Stevens-Johnson syndrome and ocular cicatricial pemphigoid, or iatrogenically after surgery, which may damage the orifices of the excretory ducts thereby reducing the volume of aqueous bathing the ocular surface [7, 15].

2.3. Contributions of the Lacrimal Gland to Ocular Surface Health. As previously suggested, the components of the tear film produced by the lacrimal gland are critical in several processes related to ocular surface health. The first is in protection of the ocular surface from invading pathogens with a local population of IgA-secreting plasma cells that reside within the lacrimal gland itself. While tear firm contains other immunoglobulins, secretory IgA is the predominant antibody and is the only immunoglobulin whose concentration significantly increases during infection, suggesting its critical role in host defense of the ocular surface [16]. The ability of the lacrimal gland to specifically select for IgA secreting plasma cells is not well understood but likely resides in the recruitment and proliferation of a specific subset of helper T cells. These T cells are recruited by an IL-2-like peptide known as lacrimal gland-derived lymphocyte proliferation potentiating factor [17, 18]. These T cells then recruit and promote B cell differentiation into IgA-secreting plasma cells. Once produced by plasma cells, dimeric IgA is translocated into the tear film by a cell surface antibody receptor to inhibit pathogen adherence to the host surface as seen at other mucosal sites [19]. The production of this translocation receptor is exquisitely sensitive to endocrine and nervous and immune system regulation [20, 21]. Consequently, the host invests significant energy into the production and secretion of IgA into the tear film to reduce ocular surface susceptibility.

The lacrimal gland also secretes several bacteria (i.e., secretory phospholipase A2, an effective antistaphylococcal enzyme among others [22]) and fungicidal agents such as lysozyme, peroxidase, tear-specific pre-albumin, psoriasin, and lactoferrin into the tear film [2, 23]. These substances greatly reduce susceptibility of the ocular surface due to cytotoxicity to invading pathogens. While it is still controversial, the lacrimal gland may also be an additional source of soluble mucin production, which acts to clear debris and hold fluid on the surface of the eye [24–26]. This glycoprotein also serves as an infectious deterrent by acting as a decoy receptor for invading pathogens [27]. As such, these cytotoxic agents, mucin, and IgA transform a susceptible, warm, moist, nutrient rich epithelial surface into

an inhospitable environment unlike other colonized mucosal surfaces.

The second major contribution of the lacrimal gland is in the aqueous produced by acinar cells that add significant volume to the tear film. The fluid is transported from the interstitial space into the lumen of the gland by way of osmosis and released onto the ocular surface [2]. The addition of high volumes of water from the gland helps to keep the ocular surface moist, maintain an important component of light refraction in the air-water-corneal interfaces, and dilute proteins within the tears to keep them solubilized. Water is also transported in conjunction with other important electrolytes required in cellular processes and has been extensively reviewed elsewhere [2]. With the addition of lipocalin and lipids from the meibomian gland, tears become a highly viscous, low surface tension solution critical in tear film stability and health of the ocular surface [28]. As such, water serves to dilute substances in the tear film and maintain an interface critical for normal visual acuity.

The lacrimal gland is also responsible for producing several other proteins and products necessary in growth and maintenance of host tissue found in the tear film. Several of these proteins are growth factors. They include epidermal, fibroblast, hepatocyte, keratinocyte, and transforming growth factor-β. While the defined role of each in corneal regeneration is unclear, these factors promote proliferation and migration of epithelial cells following disruption of the corneal surface and maintain an avascular cornea necessary for transparency of the tissue [29–34]. If these factors decline or are replaced for others, neovascularization of the cornea ensues [35, 36].

Retinol, a vitamin A derivative, is also secreted by the lacrimal gland. Retinol is required in maintenance of goblet cells within the conjunctiva and controls corneal epithelial desquamation, keratinization, and metaplasia [37–39]. Vitamin A is also a positive feedback molecule as its deficiency results in a decrease in flow rate of lacrimal gland fluid in rabbits [40]. In humans, vitamin A deficiency can result in corneal ulcers, melt, and even perforation [41]. This loss of corneal integrity is felt to be the result of an increased risk of infection, decreased tear film, alterations in corneal wound healing, and changes in leukocyte function [42]. Consequently, the ocular surface role of secreted vitamin A from the lacrimal gland is multifactorial. The previously mentioned products of the lacrimal gland are only a select few of the known proteins in the tear film and there are likely several unidentified proteins at this point in time.

In summary, the lacrimal gland secretes a complex aqueous milieu rich in antibodies, cytotoxic agents, and growth factors onto the ocular surface to protect the cornea from desiccation, infection, and vascularization while promoting wound healing and transparency.

2.4. Disease of the Lacrimal Gland. Dysfunction of the lacrimal gland may result from inflammation, aging, radiation, or infection. The end result of many of these pathologies rests in insufficient tear production and changes in osmolality and increased osmotic stress of the ocular surface [43]. This results in increased susceptibility of the ocular surface that we hypothesize is due to the loss of the previously mentioned antimicrobial tear film products [44]. Unfortunately, in inflammatory dry eye, this is further exacerbated by relatively high concentrations of proteins within the tears that induce apoptosis of surface epithelium and a vicious, self-perpetuated cycle of increased expression of proinflammatory cytokines from the ocular surface [45, 46]. The proinflammatory state further worsens dry eye by leading to apoptosis and decreased mucin production from conjunctival goblet cells [47, 48]. Matrix metalloproteinases (MMPs), a family of proteins required in wound healing and degradation of extracellular matrix, are one such proinflammatory product highly expressed in dry eye conditions and known to cause epithelial barrier dysfunction [45, 49]. As such, tests such as InflammaDry by Rapid Pathogen Screening have been developed to evaluate tear concentrations of MMPs as surrogates for inflammation in the clinical realm [50].

In the following section, we have focused on specific diseases to highlight the major causes of lacrimal dysfunction, that is, Sjogren's syndrome (SS) as a representative for inflammation (Table 1). Several diseases cause multiple types of pathology making gross categorization difficult.

2.5. Aging. Aging takes a toll on the entire body and the lacrimal gland is no different resulting in decreased tear production with increasing age [51]. Progressive acinar atrophy and fibrosis and lymphocytic infiltrates are more common within the lacrimal glands of the elderly [52]. While the exact pathophysiological changes are not well understood, mice lacking a major antioxidant pathway have been shown to have more extensive acinar atrophy and a larger leukocyte infiltrate within the lacrimal gland compared to controls [53]. Furthermore, there is likely some component of autoimmune-driven destruction of the gland with aging as $CD4^+$ T cell adoptive transfers from elderly mice into naïve, immunodeficient recipients that results in a reduction of goblet cells and T cell infiltrate into the lacrimal gland. Unfortunately, this study did not correlate pathology of the gland with this immune infiltrate [54]. As such, the exact role of this T cell infiltrate into the lacrimal gland of the elderly is undefined; however, speculation would surmise that this may result in an inflammatory dry eye disease process with lacrimal gland destruction similar to SS. This is supported in part in that the tear film of older mice contains higher concentrations of pro-inflammatory cytokines than younger mice [55]. In humans this is further supported by the upregulation of inflammatory markers with decreased aqueous production in the elderly [56]. In total, lacrimal gland hypofunction in the elderly is likely the result of oxidative damage and an ongoing autoimmune, inflammatory event.

2.6. Inflammatory Diseases of the Lacrimal Gland. SS is a systemic, chronic inflammatory state of the exocrine glands predominately seen in women that results in dry eyes and mouth. The initiating environmental factor or pathogen trigger for glandular inflammation defining the disease is unknown. A lymphocytic infiltrate, predominately activated

Table 1: Causes of lacrimal gland dysfunction and their proposed pathological mechanism. Grosscategorization of the most common causes of lacrimal gland dysfunction based on underlying pathology most typical of the disease. HIV, human immunodeficiency virus; CMV, cytomegalovirus.

Pathological changes	Disease
Inflammatory/Oxidative Stress	Sjogren's syndrome
	IgG4-related disease
	Autoimmune Dacryadenitis
	Sarcoidosis
	Chronic graft-versus host
	Thyroid disease
	Orbital inflammatory pseudotumor
	Amyotrophic Lateral Sclerosis
	Diabetes
	Aging
Infectious	HIV
	CMV
	Hepatitis C
Atrophy	Aging
	Radiation
Toxicity	Radiation
Environmental	Smoking
	Video displays
Autonomic Dysfunction	Riley-Day syndrome
Idiopathic	

$CD4^+$ T cells, is responsible for the enlargement and permanent damage of the exocrine glands resulting in reduced secretions and breakdown of mucosal surfaces [57–59]. In regard to the lacrimal gland itself, imaging studies have shown an accelerated fat deposition within the gland during SS and histopathologic changes such as intralobular fibrosis and a disorganized arrangement of the ducts occurs in even mild cases [60, 61]. Furthermore, inflammation involving the lacrimal ducts likely complicates aqueous outflow but little is known on the subject. The role of each of these changes in the overall reduction in tear production is still debatable as the degree of tissue destruction and lymphocytic infiltrate does not correlate with the level of gland dysfunction [62–64].

Despite extensive research, the exact pathophysiology of the disease remains unclear. What is clear, however, is that the tear film of patients with SS contains an inflammatory proteomic profile compared to normal controls [65]. This presumably results in epithelial decompensation and loss of goblet cells as previously described resulting in severe dry eye. In mouse models, the lacrimal and submandibular glands are the first affected in the disease process, and MMPs and other proinflammatory cytokines are upregulated in tear film [66–68]. To make matters worse, dry conditions trigger significant production of proinflammatory mediators in SS patient's within hours of introduction into the environment suggesting a frailty of the tissue [46]. As the disease progresses, lacrimal gland production wanes necessitating increased ocular lubrication and the addition of topical anti-inflammatories such as cyclosporine [69].

While the mechanism is likely similar to SS with an abnormal immune response, it is worth at least mentioning a fairly new entity, IgG4-related disease, that can cause lacrimal gland dysfunction and is a current, popular topic in the clinical and scientific realm [70, 71]. The disease is characterized by an infiltration of IgG4-producing plasma cells, elevated serum IgG4, and fibrosis and enlargement of multiple organs and was previously known under the eponym Milkulicz's disease [70, 72]. These changes within the lacrimal gland can induce dry eye. Consequently, IgG4-related disease is a known inflammatory disorder of the lacrimal gland but not as well understood as that of SS.

2.7. Environmental: Smoking and Video Displays. Smoking and video displays have been implicated in lacrimal gland dysfunction [73, 74]. While the mechanism of gland dysfunction is unclear in both, cytochrome P450s and signals of oxidative damage are upregulated in the lacrimal glands of rats exposed to cigarette smoke [73]. We hypothesize that this likely results in destruction of the gland as seen with an aging lacrimal gland; however, no study has specifically evaluated the underlying pathophysiology. In regard to video displays, lacrimal gland hypofunction and decreased tear production are dependent on the amount of time the monitor is used at work. Unfortunately untested, the authors speculate that proper lacrimal gland function is dependent on number of eyelid blinks [74]. To partially support this, patients with Parkinson's disease have poor blink rates, tear meniscus heights, and dry eye [75]. Whether blink rate and subsequent lacrimal gland hypofunction is a contributor of dry eye in Parkinson's disease is unknown, however. Regrettably, environmental causes of lacrimal gland dysfunction are poorly understood and there are likely many other factors responsible for decreased tear production that have not been identified.

2.8. Infectious: HIV. Dry eye is more prevalent in HIV patients than in the general public with a study reporting more than 85% of these patients to have findings consistent with dry eye [76, 77]. A portion of patients clearly show a reduction in tear production [78] and this is hypothesized to be due to a lymphocytic infiltrate similar to SS [79]. This is most evident in those HIV-infected patients who develop diffuse infiltrative lymphocytosis syndrome, a rare entity since the introduction of HAART. In these patients, the salivary and lacrimal glands enlarge with $CD8^+$ lymphocytes [79]. As such, lacrimal gland dysfunction during infectious diseases is likely a similar pathophysiological event as that found in SS. Therapeutic options are few as topical cyclosporine suppresses local immunity and is likely a poor choice for this case of inflammatory dry eye.

2.9. Radiation. Many head and neck cancers are treated with surgical and/or radiation therapy. While radiation is an effective treatment of rapidly dividing cancerous cells, this therapy

has well known toxic effects on local and regional tissues resulting in side effects reviewed extensively elsewhere [80]. Despite the glands of the head being highly differentiated and slowly dividing tissues, they are exquisitely sensitive to radiation that can cause transient and/or permanent dysfunction of the gland [81, 82]. Xerostomia is the most common presentation of glandular dysfunction of the head and neck; however, the lacrimal gland is also affected by radiation [83]. In rabbits, loss of smooth muscle and decreased aqueous secretion occur within 3 days of irradiation of the lacrimal gland and persist beyond thirty days [84]. Unfortunately, the long-term histopathological effects of radiation on the lacrimal gland have been poorly studied in animals and humans. In patients receiving local radiation, lacrimal gland dysfunction results in a dose-dependent increase in severity of dry eye following radiation treatment [85]. Consequently, pathological changes occur within days of radiation therapy inducing both temporary and permanent lacrimal gland dysfunction and resultant dry eye. While dry eyes are an unfortunate side effect, radiation therapy of head, neck, and orbit remains a commonly used treatment modality due to its success in treating such tumors making radiation-induced dry eye an issue for the foreseeable future [80].

2.10. Idiopathic. Lastly, there are idiopathic causes of lacrimal gland dysfunction that cannot be linked to any specific cause that may represent subclinical presentations of those previously mentioned above or an altogether undefined entity.

3. Historical Treatment of Dry Eye Related to Lacrimal Gland Dysfunction

Regrettably, the treatment of dry eye related to tear film insufficiency has made little progress in recent years. Most current therapies aim to reduce drainage of tears from the eye, that is, punctual occlusion with cautery or plugs, or to replace insufficient aqueous production from the lacrimal gland with artificial tears. Each of these therapies reduces dry eye symptoms but each has significant drawbacks. For example, punctal plugs have poor retention rates; can migrate into the lacrimal system; predispose the eye to infection; and cause epiphora [86]. Punctal cautery can cause similar issues but is much more difficult to reverse with patient intolerance. Artificial tears are a more benign therapeutic option but the preservatives within them can be toxic to the cornea with frequent dosing [87]. This issue has been circumvented by the production of preservative-free preparations. While the ingredients have significantly changed, artificial tears were first described nearly 3,500 years ago and are unfortunately rapidly removed from the ocular surface [88]. It was not until the 1980s that natural or synthetic polymers were added to preparations increasing viscosity and retention time. Such compounds as 1% glycerin have shown prolonged benefit compared to propylene and polyethylene glycol are one such example [89]. Even with these advancements, artificial tears are only a temporary measure and do not provide important proteins produced by the lacrimal gland for ocular health as previously discussed and are short-lived. As such, artificial tears and punctal occlusion remain viable options for dry eye treatment but do not address the underlying lacrimal gland dysfunction.

Further advancements have been made with the introduction of cyclosporine. The compound was initially isolated from the fungus *Tolypocladium inflatum*, a potent inhibitor of T cell activity [90, 91]. Therapy with this medication has shown great effect by increasing goblet cell density and TGF-β. It has also been shown in mice to better reduce epithelial staining compared to prednisone in an inflammatory dry eye model [92]. However, this therapy is presumably most effective in an inflammatory dry eye minimizing its therapeutic use to these specific conditions. Additionally, poor patient compliance further reduces its widespread application due to ocular irritation and prolonged use necessary to see any appreciable benefit frustrating even the most compliant patients.

Multiple surgical attempts have been made to bypass the lacrimal gland altogether by transposing the parotid gland duct onto the lower conjunctiva, a technique developed in the 1950s [93]. This has shown great promise in dogs with keratoconjunctivitis sicca with a success rate as high as 92% [94], but the results are difficult to interpret in animals unable to voice complaints of dry eye or excessive tearing. We, as well as others, have all but abandoned the technique due to lack of any appreciable benefit, excessive tear secretion, and high rates atrophy of the gland following surgery [95]. Consequently, this technique is rarely used today except in animals due to inconsistent results and significant side effects.

4. Emerging Therapies for Dry Eye

There are several emerging modalities that have shown at least some promise in the basic science realm. These emerging therapies can be divided into two categories: exogenous compounds and gland regeneration/bypass. Unfortunately, many of these treatments are still in their infancy and have not made significant progress beyond the basic science realm.

Exogenous compounds are delivered to host tissue through either topical or oral routes. Many of these new agents directly inhibit proinflammatory cascades. The list of targets includes vascular cell adhesion inhibitors, immune modulators, and immune suppressants [96–98]. These inhibitors have shown efficacy in mouse models of inflammatory dry eye but have not been used in humans. One of these agents is delivered in an adenoviral vector, which would theoretically reduce the need for reapplication but raises concerns for inducing an innate immune response that could worsen inflammation [35, 98]. Moreover, immune suppression of the ocular surface could result in frequent infectious complications. The recently described nonimmune compound, pituitary adenylate cyclase-activating polypeptide-derived peptide, has been shown to promote corneal wound healing and lacrimal gland secretion in mice [99]. With the adenoviral vector as the exception, these compounds would likely be an improvement from artificial tears but would reduce ocular immunity. Patient compliance as seen with other topical eye medications would also be an issue.

Consequently, the untested role of these topical therapies may be of some benefit in few select populations.

With increasing success in stem cell-based tissue regeneration, tissues and organs such as functioning photoreceptors and the liver can now be grown *in vitro* [100, 101]. Attempts of a bioengineered lacrimal gland have seen recent success in mouse models as well [102]. Stem cells are isolated using specific lacrimal cell markers, tissue grown *ex vivo*, and transplanted into the host resulting in increased tear production [102–104]. While promising, it remains to be seen whether these results can be reproduced in humans and provide a feasible, long lasting therapeutic option. There has also been some suggestion of using lacrimal gland xenografts as healthy tissue, but this theoretical idea remains untested [105]. Furthermore, these transplant models have not evaluated the effect of transplantation with ongoing diseases such as SS that may reduce graft transplantation rates and efficacy such as seen with a significantly higher rejection rate of herpes-infected corneas compared to noninflammatory corneal transplants [106]. While clinical promising and would address gland dysfunction and restore normal tear production, lacrimal gland regeneration or xenograph transplantation remains to be years from clinical use.

More recently, sublingual, labial, and submandibular glands have been transplanted into the subconjunctival space as an additional means to treat severe dry eyes due to underlying basal secretion of these glands that does not require innervation [107, 108]. The transplanted glands have shown a reduction in dry eye symptoms for at least five years and reduce the need for tear supplementation [109]. In addition, saliva contains many of the same contents as the lacrimal gland including secretory IgA but the two have not been specifically compared [110]. Unfortunately, the long-term efficacy beyond 5 years is currently unknown. Furthermore, the transplantation rate is at best 72% and requires a difficult microsurgery including vascular anastomosis of the gland to the temporal artery and vein [111]. As such, the surgery has not gained widespread use at this point in time. The transplants can also cause chronic inflammation exacerbating dry eye symptoms, microcystic epithelial edema, and epiphora in approximately 40% of patients within 3–6 months of surgery [112, 113]. While transplantation of these glands has shown great effect on dry eye and produce more natural tears than artificial instilled ones, the complicated surgery and risk of graft failure are staggering complications to overcome making them less than ideal.

Lastly, there is ongoing work on an implantable device to that stimulates the lacrimal nerve to increase tear production within the lacrimal gland and this small animal study has shown promising results [114]. However, it remains to be seen whether this method of hyperstimulating the lacrimal gland can and/or will overcome gland dysfunction and whether it becomes a feasible clinical treatment option. Furthermore, will this device be able to stimulate a diseased gland enough such as in SS to overcome the symptoms of dry eye?

In summary, there are several new modalities emerging for severe dry eye; however, many of these options remain unproven or require extensive, technically difficult microsurgery.

5. Conclusion

There are multiple disease entities that can affect the lacrimal gland and cause its dysfunction. Untreated pathologies and downstream effects of reduced production from the lacrimal gland can result in decompensation of the ocular surface and gross deterioration of visual acuity. The role of this gland cannot be overstated in ocular surface health and proper light refraction from the air-tear interface. Emerging therapies will hopefully alleviate the large dry eye burden worldwide by addressing the issue at its core, by attempting to regenerate a dysfunctional gland and/or controlling the proinflammatory state that ensues with severe dry eye. As such, new modalities and therapies need to be developed through collaborative/translational research to treat aqueous deficiency-related dry eye. It will be interesting to see if the untested, but promising, therapies discussed become viable treatment modalities in dry eye therapy beyond the temporary measures of ocular lubricants.

Conflict of Interests

The authors have no conflict of interests to declare.

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References

[1] T. J. Millar and B. S. Schuett, "The real reason for having a meibomian lipid layer covering the outer surface of the tear film—a review," *Experimental Eye Research*, vol. 137, pp. 125–138, 2015.

[2] B. Walcott, "The lacrimal gland and its veil of tears," *News in Physiological Sciences*, vol. 13, no. 2, pp. 97–103, 1998.

[3] A. J. Bron and J. M. Tiffany, "The contribution of meibomian disease to dry eye," *Ocular Surface*, vol. 2, no. 2, pp. 149–164, 2004.

[4] P. S. Tsai, J. E. Evans, K. M. Green et al., "Proteomic analysis of human meibomian gland secretions," *British Journal of Ophthalmology*, vol. 90, no. 3, pp. 372–377, 2006.

[5] F. A. Mausolf, *The Anatomy of the Ocular Adnexa; Guide to Orbital Dissection*, Thomas, Springfield, Ill, USA, 1975.

[6] S. E. Whitnall, "Anatomy of the human orbit and accessory organs of vision," in *Classics in Ophthalmology*, Krieger, Huntington, NY, USA, 1979.

[7] H. Obata, "Anatomy and histopathology of the human lacrimal gland," *Cornea*, vol. 25, no. 10, supplement 1, pp. S82–S89, 2006.

[8] "Basic and clinical science course: orbit, eyelids, and lacrimal system," *American Academy of Ophthalmoloy*, vol. 7, pp. 243–244, 2012.

[9] C. M. Riley, R. C. Day, D. M. Greeley, and W. S. Langford, "Central autonomic dysfunction with defective lacrimation; report of five cases," *Pediatrics*, vol. 3, no. 4, pp. 468–478, 1949.

[10] L. T. Jones, "The lacrimal secretory system and its treatment," *American Journal of Ophthalmology*, vol. 62, no. 1, pp. 47–60, 1966.

[11] H. Obata, S. Yamamoto, H. Horiuchi, and R. Machinami, "Histopathologic study of human lacrimal gland: statistical analysis with special reference to aging," *Ophthalmology*, vol. 102, no. 4, pp. 678–686, 1995.

[12] J. L. Roen, O. G. Stasior, and F. A. Jakobiec, "Aging changes in the human lacrimal gland: role of the ducts," *CLAO Journal*, vol. 11, no. 3, pp. 237–242, 1985.

[13] M. R. Allansmith, G. Kajiyama, M. B. Abelson, and M. A. Simon, "Plasma cell content of main and accessory lacrimal glands and conjunctiva," *American Journal of Ophthalmology*, vol. 82, no. 6, pp. 819–826, 1976.

[14] Y. Ogawa, K. Yamazaki, M. Kuwana et al., "A significant role of stromal fibroblasts in rapidly progressive dry eye in patients with chronic GVHD," *Investigative Ophthalmology and Visual Science*, vol. 42, no. 1, pp. 111–119, 2001.

[15] B. Smith and R. Petrelli, "Surgical repair of prolapsed lacrimal glands," *Archives of Ophthalmology*, vol. 96, no. 1, pp. 113–114, 1978.

[16] D. K. Sen and G. S. Sarin, "Immunoglobulin concentrations in human tears in ocular diseases," *British Journal of Ophthalmology*, vol. 63, no. 5, pp. 297–300, 1979.

[17] S. H. Liu, D.-H. Zhou, and R. M. Franklin, "Lacrimal gland-derived lymphocyte proliferation potentiating factor," *Investigative Ophthalmology and Visual Science*, vol. 34, no. 3, pp. 650–657, 1993.

[18] R. M. Franklin and K. F. Shepard, "T-cell adherence to lacrimal gland: the event responsible for IgA plasma cell predominance in lacrimal gland," *Regional Immunology*, vol. 3, no. 4, pp. 213–216, 1990.

[19] A. J. Macpherson, D. Gatto, E. Sainsbury, G. R. Harriman, H. Hengartner, and R. M. Zinkernagel, "A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria," *Science*, vol. 288, no. 5474, pp. 2222–2226, 2000.

[20] R. S. Kelleher, L. E. Hann, J. A. Edwards, and D. A. Sullivan, "Endocrine, neural, and immune control of secretory component output by lacrimal gland acinar cells," *Journal of Immunology*, vol. 146, no. 10, pp. 3405–3412, 1991.

[21] D. A. Sullivan and L. E. Hann, "Hormonal influence on the secretory immune system of the eye: endocrine impact on the lacrimal gland accumulation and secretion of IgA and IgG," *Journal of Steroid Biochemistry*, vol. 34, no. 1–6, pp. 253–262, 1989.

[22] X.-D. Qu and R. I. Lehrer, "Secretory phospholipase A2 is the principal bactericide for staphylococci and other gram-positive bacteria in human tears," *Infection and Immunity*, vol. 66, no. 6, pp. 2791–2797, 1998.

[23] F. Garreis, M. Gottschalt, T. Schlorf et al., "Expression and regulation of antimicrobial peptide psoriasin (S100A7) at the ocular surface and in the lacrimal apparatus," *Investigative Ophthalmology and Visual Science*, vol. 52, no. 7, pp. 4914–4922, 2011.

[24] M. M. Jumblatt, R. W. McKenzie, P. S. Steele, C. G. Emberts, and J. E. Jumblatt, "MUC7 expression in the human lacrimal gland and conjunctiva," *Cornea*, vol. 22, no. 1, pp. 41–45, 2003.

[25] M. E. Arango, P. Li, M. Komatsu, C. Montes, C. A. C. Carraway, and K. L. Carraway, "Production and localization of Muc4/Sialomucin complex and its receptor tyrosine kinase ErbB2 in the rat lacrimal gland," *Investigative Ophthalmology & Visual Science*, vol. 42, no. 12, pp. 2749–2756, 2001.

[26] I. K. Gipson, "Distribution of mucins at the ocular surface," *Experimental Eye Research*, vol. 78, no. 3, pp. 379–388, 2004.

[27] D. A. Dartt and M. D. P. Willcox, "Complexity of the tear film: importance in homeostasis and dysfunction during disease," *Experimental Eye Research*, vol. 117, pp. 1–3, 2013.

[28] A. J. Bron, J. M. Tiffany, S. M. Gouveia, N. Yokoi, and L. W. Voon, "Functional aspects of the tear film lipid layer," *Experimental Eye Research*, vol. 78, no. 3, pp. 347–360, 2004.

[29] J. Imanishi, K. Kamiyama, I. Iguchi, M. Kita, C. Sotozono, and S. Kinoshita, "Growth factors: importance in wound healing and maintenance of transparency of the cornea," *Progress in Retinal and Eye Research*, vol. 19, no. 1, pp. 113–129, 2000.

[30] K. Yoshino, R. Garg, D. Monroy, Z. Ji, and S. C. Pflugfelder, "Production and secretion of transforming growth factor beta (TGF-β) by the human lacrimal gland," *Current Eye Research*, vol. 15, no. 6, pp. 615–624, 1996.

[31] S. E. Wilson, S. A. Lloyd, and R. H. Kennedy, "Epidermal growth factor messenger RNA production in human lacrimal gland," *Cornea*, vol. 10, no. 6, pp. 519–524, 1991.

[32] S. E. Wilson, S. A. Lloyd, and R. H. Kennedy, "Basic fibroblast growth factor (FGFb) and epidermal growth factor (EGF) receptor messenger RNA production in human lacrimal gland," *Investigative Ophthalmology and Visual Science*, vol. 32, no. 10, pp. 2816–2820, 1991.

[33] Q. Li, J. Weng, R. R. Mohan et al., "Hepatocyte growth factor and hepatocyte growth factor receptor in the lacrimal gland, tears, and cornea," *Investigative Ophthalmology and Visual Science*, vol. 37, no. 5, pp. 727–739, 1996.

[34] S. E. Wilson, Q. Liang, and W. J. Kim, "Lacrimal gland HGF, KGF, and EGF mRNA levels increase after corneal epithelial wounding," *Investigative Ophthalmology & Visual Science*, vol. 40, no. 10, pp. 2185–2190, 1999.

[35] K. Bryant-Hudson, C. D. Conrady, and D. J. J. Carr, "Type I interferon and lymphangiogenesis in the HSV-1 infected cornea—are they beneficial to the host?" *Progress in Retinal and Eye Research*, vol. 36, pp. 281–291, 2013.

[36] C. D. Conrady, M. Zheng, D. U. Stone, and D. J. J. Carr, "$CD8^+$ T cells suppress viral replication in the cornea but contribute to VEGF-C-induced lymphatic vessel genesis," *The Journal of Immunology*, vol. 189, no. 1, pp. 425–432, 2012.

[37] R. R. Pfister and M. E. Renner, "The corneal and conjunctival surface in vitamin A deficiency: a scanning electron microscopy study," *Investigative Ophthalmology and Visual Science*, vol. 17, no. 9, pp. 874–883, 1978.

[38] Y. Zhang, Z. Liu, X. Chen, S. Zhang, D. Yu, and C. Li, "The effect of oculotect gel on the conjunctival goblet cells of rat after corneal transplantation," *Yan Ke Xue Bao*, vol. 22, no. 4, pp. 229–243, 2006.

[39] V. Rao, J. Friend, R. A. Thoft, B. A. Underwood, and P. R. Reddy, "Conjunctival goblet cells and mitotic rate in children with retinol deficiency and measles," *Archives of Ophthalmology*, vol. 105, no. 3, pp. 378–380, 1987.

[40] V. Rismondo, T. B. Osgood, P. Leering, M. G. Hattenhauer, J. L. Ubels, and H. F. Edelhauser, "Electrolyte composition of lacrimal gland fluid and tears of normal and vitamin A-deficient rabbits," *The CLAO Journal*, vol. 15, no. 3, pp. 222–228, 1989.

[41] A. P. Lange, G. Moloney, C. A. Sheldon, S. Sasaki, and S. P. Holland, "Bilateral corneal ulceration caused by vitamin A deficiency in eosinophilic gastroenteropathy," *Case Reports in Ophthalmology*, vol. 2, no. 3, pp. 302–306, 2011.

[42] S. S. Twining, X. Zhou, D. P. Schulte, P. M. Wilson, B. Fish, and J. Moulder, "Effect of vitamin A deficiency on the early response to experimental Pseudomonas keratitis," *Investigative Ophthalmology and Visual Science*, vol. 37, no. 4, pp. 511–522, 1996.

[43] M. Schargus, S. Ivanova, V. Kakkassery, H. B. Dick, and S. Joachim, "Correlation of tear film osmolarity and 2 different MMP-9 tests with common dry eye tests in a cohort of non-dry eye patients," *Cornea*, vol. 34, no. 7, pp. 739–744, 2015.

[44] E. V. Boiko, A. L. Pozniak, D. S. Maltsev, A. A. Suetov, and I. V. Nuralova, "High frequency of latent conjunctival *C. trachomatis*, *M. hominis*, and *U. urealyticum* infections in young adults with dry eye disease," *Journal of Ophthalmology*, vol. 2014, Article ID 154627, 7 pages, 2014.

[45] L. Luo, D.-Q. Li, and S. C. Pflugfelder, "Hyperosmolarity-induced apoptosis in human corneal epithelial cells is mediated by cytochrome c and MAPK pathways," *Cornea*, vol. 26, no. 4, pp. 452–460, 2007.

[46] A. López-Miguel, M. Tesón, V. Martín-Montañez et al., "Clinical and molecular inflammatory response in sjögren syndrome-associated dry eye patients under desiccating stress," *American Journal of Ophthalmology*, vol. 161, pp. 133–141.e2, 2016.

[47] I. C. You, F. Bian, E. A. Volpe, C. S. de Paiva, and S. C. Pflugfelder, "Age-related conjunctival disease in the C57BL/6.NOD-Aec1Aec2 Mouse Model of Sjögren Syndrome develops independent of lacrimal dysfunction," *Investigative Ophthalmology & Visual Science*, vol. 56, no. 4, pp. 2224–2233, 2015.

[48] L. Contreras-Ruiz, A. Ghosh-Mitra, M. A. Shatos, D. A. Dartt, and S. Masli, "Modulation of conjunctival goblet cell function by inflammatory cytokines," *Mediators of Inflammation*, vol. 2013, Article ID 636812, 11 pages, 2013.

[49] S. C. Pflugfelder, W. Farley, L. Luo et al., "Matrix metalloproteinase-9 knockout confers resistance to corneal epithelial barrier disruption in experimental dry eye," *The American Journal of Pathology*, vol. 166, no. 1, pp. 61–71, 2005.

[50] H. E. Kaufman, "The practical detection of MMP-9 diagnoses ocular surface disease and may help prevent its complications," *Cornea*, vol. 32, no. 2, pp. 211–216, 2013.

[51] A. J. Paulsen, K. J. Cruickshanks, M. E. Fischer et al., "Dry eye in the beaver dam offspring study: prevalence, risk factors, and health-related quality of life," *American Journal of Ophthalmology*, vol. 157, no. 4, pp. 799–806, 2014.

[52] E. M. Rocha, M. Alves, J. D. Rios, and D. A. Dartt, "The aging lacrimal gland: changes in structure and function," *Ocular Surface*, vol. 6, no. 4, pp. 162–174, 2008.

[53] T. Kojima, T. H. Wakamatsu, M. Dogru et al., "Age-related dysfunction of the lacrimal gland and oxidative stress: evidence from the Cu,Zn-superoxide dismutase-1 (Sod1) knockout mice," *The American Journal of Pathology*, vol. 180, no. 5, pp. 1879–1896, 2012.

[54] A. J. McClellan, E. A. Volpe, X. Zhang et al., "Ocular surface disease and dacryoadenitis in aging C57BL/6 mice," *The American Journal of Pathology*, vol. 184, no. 3, pp. 631–643, 2014.

[55] K. S. Krabbe, M. Pedersen, and H. Bruunsgaard, "Inflammatory mediators in the elderly," *Experimental Gerontology*, vol. 39, no. 5, pp. 687–699, 2004.

[56] A. Tomlinson, S. Khanal, K. Ramaesh, C. Diaper, and A. McFadyen, "Tear film osmolarity: determination of a referent for dry eye diagnosis," *Investigative Ophthalmology and Visual Science*, vol. 47, no. 10, pp. 4309–4315, 2006.

[57] C. Vitali, S. Bombardieri, R. Jonsson et al., "Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group," *Annals of the Rheumatic Diseases*, vol. 61, no. 6, pp. 554–558, 2002.

[58] S. Cha, A. B. Peck, and M. G. Humphreys-Beher, "Progress in understanding autoimmune exocrinopathy using the non-obese diabetic mouse: an update," *Critical Reviews in Oral Biology and Medicine*, vol. 13, no. 1, pp. 5–16, 2002.

[59] R. I. Fox, H.-I. Kang, D. Ando, J. Abrams, and E. Pisa, "Cytokine mRNA expression in salivary gland biopsies of Sjogren's syndrome," *Journal of Immunology*, vol. 152, no. 11, pp. 5532–5539, 1994.

[60] J. Williamson, A. A. M. Gibson, T. Wilson, J. V. Forrester, K. Whaley, and W. C. Dick, "Histology of the lacrimal gland in keratoconjunctivitis sicca," *British Journal of Ophthalmology*, vol. 57, no. 11, pp. 852–858, 1973.

[61] M. Izumi, K. Eguchi, M. Uetani et al., "MR features of the lacrimal gland in Sjogren's syndrome," *American Journal of Roentgenology*, vol. 170, no. 6, pp. 1661–1666, 1998.

[62] Y. Paranyuk, N. Claros, A. Birzgalis, L. C. Moore, P. R. Brink, and B. Walcott, "Lacrimal gland fluid secretion and lymphocytic infiltration in the NZB/W mouse model of Sjögren's syndrome," *Current Eye Research*, vol. 23, no. 3, pp. 199–205, 2001.

[63] M. V. Jonsson, N. Delaleu, K. A. Brokstad, E. Berggreen, and K. Skarstein, "Impaired salivary gland function in NOD mice: association with changes in cytokine profile but not with histopathologic changes in the salivary gland," *Arthritis & Rheumatism*, vol. 54, no. 7, pp. 2300–2305, 2006.

[64] U. S. Deshmukh, Y. Ohyama, H. Bagavant, X. Guo, F. Gaskin, and S. M. Fu, "Inflammatory stimuli accelerate Sjogren's syndrome-like disease in (NZB × NZW)F_1 mice," *Arthritis & Rheumatism*, vol. 58, no. 5, pp. 1318–1323, 2008.

[65] B. Li, M. Sheng, J. Li et al., "Tear proteomic analysis of Sjögren syndrome patients with dry eye syndrome by two-dimensional-nano-liquid chromatography coupled with tandem mass spectrometry," *Scientific Reports*, vol. 4, article 5772, 2014.

[66] J. Xuan, L. Shen, K. Malyavantham, O. Pankewycz, J. L. Ambrus Jr., and L. Suresh, "Temporal histological changes in lacrimal and major salivary glands in mouse models of Sjogren's syndrome," *BMC Oral Health*, vol. 13, article 51, 2013.

[67] H. S. Aluri, C. L. Kublin, S. Thotakura et al., "Role of matrix metalloproteinases 2 and 9 in lacrimal gland disease in animal models of Sjögren's syndrome," *Investigative Opthalmology & Visual Science*, vol. 56, no. 9, pp. 5218–5128, 2015.

[68] C. P. Robinson, J. Cornelius, D. E. Bounous, H. Yamamoto, M. G. Humphreys-Beher, and A. B. Peck, "Characterization of the changing lymphocyte populations and cytokine expression in the exocrine tissues of autoimmune NOD mice," *Autoimmunity*, vol. 27, no. 1, pp. 29–44, 1998.

[69] M. Ramos-Casals, A. G. Tzioufas, J. H. Stone, A. Sisó, and X. Bosch, "Treatment of primary Sjögren syndrome: a systematic review," *The Journal of the American Medical Association*, vol. 304, no. 4, pp. 452–460, 2010.

[70] H. Hamano, S. Kawa, A. Horiuchi et al., "High serum IgG4 concentrations in patients with sclerosing pancreatitis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 732–738, 2001.

[71] L. Vasaitis, "IgG4-related disease: a relatively new concept for clinicians," *European Journal of Internal Medicine*, vol. 27, pp. 1–9, 2016.

[72] M. Yamamoto, H. Yajima, H. Takahashi et al., "Everyday clinical practice in IgG4-related dacryoadenitis and/or sialadenitis: results from the SMART database," *Modern Rheumatology*, vol. 25, no. 2, pp. 199–204, 2015.

[73] A. Higuchi, K. Ito, M. Dogru et al., "Corneal damage and lacrimal gland dysfunction in a smoking rat model," *Free Radical Biology and Medicine*, vol. 51, no. 12, pp. 2210–2216, 2011.

[74] S. Nakamura, S. Kinoshita, N. Yokoi et al., "Lacrimal hypofunction as a new mechanism of dry eye in visual display terminal users," *PLoS ONE*, vol. 5, no. 6, Article ID e11119, 2010.

[75] C. Tamer, I. M. Melek, T. Duman, and H. Öksüz, "Tear film tests in Parkinson's disease patients," *Ophthalmology*, vol. 112, no. 10, pp. 1795.e1–1795.e8, 2005.

[76] J. A. Lucca, R. L. Farris, L. Bielory, and A. R. Caputo, "Keratoconjunctivitis sicca in male patients infected with human immunodeficiency virus type 1," *Ophthalmology*, vol. 97, no. 8, pp. 1008–1010, 1990.

[77] T. Burtin, N. Guepratte, J. L. Bourges, C. Garcher, P. Le Hoang, and C. Baudouin, "Abnormalities of the ocular surface in patients with AIDS," *Journal Français d'Ophtalmologie*, vol. 21, no. 9, pp. 637–642, 1998.

[78] S. A. Geier, S. Libera, V. Klauss, and F. D. Goebel, "Sicca syndrome in patients infected with the human immunodeficiency virus," *Ophthalmology*, vol. 102, no. 9, pp. 1319–1324, 1995.

[79] M. Alves, R. N. Angerami, and E. M. Rocha, "Dry eye disease caused by viral infection: review," *Arquivos Brasileiros de Oftalmologia*, vol. 76, no. 2, pp. 129–132, 2013.

[80] T. Y. Seiwert, J. K. Salama, and E. E. Vokes, "The chemoradiation paradigm in head and neck cancer," *Nature Clinical Practice Oncology*, vol. 4, no. 3, pp. 156–171, 2007.

[81] R. Solans, J. A. Bosch, P. Galofré et al., "Salivary and lacrimal gland dysfunction (Sicca syndrome) after radioiodine therapy," *Journal of Nuclear Medicine*, vol. 42, no. 5, pp. 738–743, 2001.

[82] O. Grundmann, G. C. Mitchell, and K. H. Limesand, "Sensitivity of salivary glands to radiation: from animal models to therapies," *Journal of Dental Research*, vol. 88, no. 10, pp. 894–903, 2009.

[83] P. Dirix, S. Nuyts, and W. Van Den Bogaert, "Radiation-induced xerostomia in patients with head and neck cancer: a literature review," *Cancer*, vol. 107, no. 11, pp. 2525–2534, 2006.

[84] S. G. Hakim, C. Schroder, G. Geerling et al., "Early and late immunohistochemical and ultrastructural changes associated with functional impairment of the lachrymal gland following external beam radiation," *International Journal of Experimental Pathology*, vol. 87, no. 1, pp. 65–71, 2006.

[85] N. Bhandare, V. Moiseenko, W. Y. Song, C. G. Morris, M. T. Bhatti, and W. M. Mendenhall, "Severe dry eye syndrome after radiotherapy for head-and-neck tumors," *International Journal of Radiation Oncology Biology Physics*, vol. 82, no. 4, pp. 1501–1508, 2012.

[86] R. Bourkiza and V. Lee, "A review of the complications of lacrimal occlusion with punctal and canalicular plugs," *Orbit*, vol. 31, no. 2, pp. 86–93, 2012.

[87] N. Onizuka, M. Uematsu, M. Kusano, H. Sasaki, K. Suzuma, and T. Kitaoka, "Influence of different additives and their concentrations on corneal toxicity and antimicrobial effect of benzalkonium chloride," *Cornea*, vol. 33, no. 5, pp. 521–526, 2014.

[88] S. R. Andersen, "The eye and its diseases in Ancient Egypt," *Acta Ophthalmologica Scandinavica*, vol. 75, no. 3, pp. 338–344, 1997.

[89] W. G. Gensheimer, D. M. Kleinman, M. O. Gonzalez et al., "Novel formulation of glycerin 1% artificial tears extends tear film break-up time compared with systane lubricant eye drops," *Journal of Ocular Pharmacology and Therapeutics*, vol. 28, no. 5, pp. 473–478, 2012.

[90] D. A. Cantrell and K. A. Smith, "The interleukin-2 T-cell system: a new cell growth model," *Science*, vol. 224, no. 4655, pp. 1312–1316, 1984.

[91] D. I. Pritchard, "Sourcing a chemical succession for cyclosporin from parasites and human pathogens," *Drug Discovery Today*, vol. 10, no. 10, pp. 688–691, 2005.

[92] Y. J. Kim, J. S. Ryu, S. Y. Park et al., "Comparison of topical application of TSG-6, cyclosporine, and prednisolone for treating dry eye," *Cornea*, 2016.

[93] J. E. Bennett and A. L. Bailey, "A surgical approach to total xerophthalmia; transplantation of the parotid duct to the inferior cul-de-sac," *AMA Archives of Ophthalmology*, vol. 58, no. 3, pp. 367–371, 1957.

[94] M. Rhodes, C. Heinrich, H. Featherstone et al., "Parotid duct transposition in dogs: a retrospective review of 92 eyes from 1999 to 2009," *Veterinary Ophthalmology*, vol. 15, no. 4, pp. 213–222, 2012.

[95] J. E. Bennett, "The management of total xerophthalmia," *Archives of Ophthalmology*, vol. 81, no. 5, pp. 667–682, 1969.

[96] T. G. Coursey, J. T. Henriksson, D. C. Marcano et al., "Dexamethasone nanowafer as an effective therapy for dry eye disease," *Journal of Controlled Release*, vol. 213, pp. 168–174, 2015.

[97] A. H. Krauss, R. M. Corrales, F. S. Pelegrino, J. Tukler-Henriksson, S. C. Pflugfelder, and C. S. de Paiva, "Improvement of outcome measures of dry eye by a novel integrin antagonist in the murine desiccating stress model," *Investigative Opthalmology & Visual Science*, vol. 56, no. 10, pp. 5888–5895, 2015.

[98] M. D. Trousdale, Z. Zhu, D. Stevenson, J. E. Schechter, T. Ritter, and A. K. Mircheff, "Expression of TNF inhibitor gene in the lacrimal gland promotes recovery of tear production and tear stability and reduced immunopathology in rabbits with induced autoimmune dacryoadenitis," *Journal of Autoimmune Diseases*, vol. 2, article 6, 2005.

[99] Y. Ma, S. Zhao, X. Wang et al., "A new recombinant PACAP-derived peptide efficiently promotes corneal wound repairing and lacrimal secretion," *Investigative Opthalmology & Visual Science*, vol. 56, no. 8, pp. 4336–4349, 2015.

[100] X. Zhong, C. Gutierrez, T. Xue et al., "Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs," *Nature Communications*, vol. 5, article 4047, 2014.

[101] T. Takebe, K. Sekine, M. Enomura et al., "Vascularized and functional human liver from an iPSC-derived organ bud transplant," *Nature*, vol. 499, no. 7459, pp. 481–484, 2013.

[102] M. Hirayama, M. Ogawa, M. Oshima et al., "Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ," *Nature Communications*, vol. 4, article 2497, 2013.

[103] P. Ackermann, S. Hetz, J. Dieckow et al., "Isolation and investigation of presumptive murine lacrimal gland stem cells," *Investigative Ophthalmology & Visual Science*, vol. 56, no. 8, pp. 4350–4363, 2015.

[104] H. Toshida, T. Ohta, C. Suto, and A. Murakami, "Effect of subconjunctival lacrimal gland transplantation in a rabbit dry eye model," *Cornea*, vol. 32, no. 11, pp. S46–S51, 2013.

[105] R. Henker, M. Scholz, S. Gaffling et al., "Morphological features of the porcine lacrimal gland and its compatibility for human

lacrimal gland xenografting," *PLoS ONE*, vol. 8, no. 9, Article ID e74046, 2013.

[106] A. Y. Chan, C. D. Conrady, K. Ding, J. D. Dvorak, and D. U. Stone, "Factors associated with age of onset of herpes zoster ophthalmicus," *Cornea*, vol. 34, no. 5, pp. 535–540, 2015.

[107] G. Geerling, P. Sieg, G.-O. Bastian, and H. Laqua, "Transplantation of the autologous submandibular gland for most severe cases of keratoconjunctivitis sicca," *Ophthalmology*, vol. 105, no. 2, pp. 327–335, 1998.

[108] D. R. Marinho, T. G. Burmann, and S. Kwitko, "Labial salivary gland transplantation for severe dry eye due to chemical burns and Stevens-Johnson syndrome," *Ophthalmic Plastic and Reconstructive Surgery*, vol. 26, no. 3, pp. 182–184, 2010.

[109] M. Borrelli, C. Schröder, J. K. G. Dart et al., "Long-term follow-up after submandibular gland transplantation in severe dry eyes secondary to cicatrizing conjunctivitis," *The American Journal of Ophthalmology*, vol. 150, no. 6, pp. 894–904, 2010.

[110] W. M. Edgar, "Saliva: its secretion, composition and functions," *British Dental Journal*, vol. 172, no. 8, pp. 305–312, 1992.

[111] G. Geerling and P. Sieg, "Transplantation of the major salivary glands," *Developments in Ophthalmology*, vol. 41, pp. 255–268, 2008.

[112] J.-Z. Su, N.-Y. Yang, X.-J. Liu et al., "Obstructive sialadenitis of a transplanted submandibular Gland: chronic inflammation secondary to ductal obstruction," *British Journal of Ophthalmology*, vol. 98, no. 12, pp. 1672–1677, 2014.

[113] G. Geerling, J. R. Garrett, K. L. Paterson et al., "Innervation and secretory function of transplanted human submandibular salivary glands," *Transplantation*, vol. 85, no. 1, pp. 135–140, 2008.

[114] A. L. Kossler, J. Wang, W. Feuer, and D. T. Tse, "Neurostimulation of the lacrimal nerve for enhanced tear production," *Ophthalmic Plastic and Reconstructive Surgery*, vol. 31, no. 2, pp. 145–151, 2015.

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Research Article

A Controlled Study on the Correlation between Tear Film Volume and Tear Film Stability in Diabetic Patients

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Purpose. To assess the tear film quantity and correlate it with the quality and stability of the tear film in diabetics and compare them to age matched controls. *Introduction*. Diabetes affects tear film parameters in multiple ways. Poor metabolic control and neuropathy are postulated factors. To further understand how diabetes affects tear film parameters this study was conducted. *Subjects and Methods*. Tear meniscus height was measured by anterior segment OCT, along with tear thinning time, a subtype of noninvasive tear break-up time, and blinking rate per minute which were all recorded for 22 diabetic patients. Correlations between these tear film parameters were studied and then compared to 16 age matched controls. *Results*. A statistically significant difference was found in blinking rate between the diabetic and the control group ($P = 0.002$), with higher blinking rate among diabetics. All tear film parameters were negatively correlated with duration of diabetes. A positive correlation was found between tear film volume and stability. *Conclusion*. Diabetes affects the tear film in various ways. Diabetics should be examined for dry eye signs even in absence of symptoms which may be masked by associated neuropathy. Duration of diabetes has an impact on tear film status.

1. Introduction

Tear film studies have progressed a lot in the past few years, from the traditional quantitative tests like Schirmer test, and tear meniscus height measurement, and the qualitative test of tear break-up time (TBUT), to anterior segment optical coherence tomography (AS OCT) measured tear film height and the dynamic tear film studies frequently used nowadays using computerized videokeratoscopy [1].

A lot of studies pointed to the fact that diabetes mellitus greatly affects the tear film function and stability. Decreased Schirmer 1 test values and shorter BUT were positively correlated with the subjective severity of dry eye symptoms in type 2 diabetic patients. Moreover, the decreased tear film function was found to be more severe in patients with PDR than in those with NPDR [2].

Other studies tested the corneal sensitivity, corneal epithelial integrity, and conjunctival epithelium (using impression cytology) and showed that the degree of keratoepitheliopathy was marked, and the corneal sensitivity, TBUT, and tear secretion were all significantly reduced in the diabetic patients. Conjunctival impression cytology showed conjunctival squamous metaplasia and lower goblet cell count in diabetic patients. All these parameters were related to the poor metabolic control, the presence of diabetic neuropathy, and the stage of diabetic retinopathy [3].

In fact, a recent study stated that tear film instability could be a marker of, or rather a predictor for, the occurrence of diabetic neuropathy in type 1 diabetes patients. Misra and coresearchers found a positive correlation between tear film stability and corneal subbasal nerve density (measured by corneal in vivo confocal microscopy) which was statistically significant ($P = 0.04$); they also found that decreased tear film stability was associated with increasing age and duration of diabetes [4].

Recent tests for assessment of the tear film quantity include AS OCT measurement of the tear wedge area, or the tear meniscus height of the inferior tear meniscus. Other tests for the assessment of tear film stability include the noninvasive BUT and its subtype, the tear thinning time (TTT), which measures the time taken for the tear film to get thinned out even before the actual break-up of the tear film occurs. The TTT has the advantage of not having to instill any eye drops or fluorescein dye prior to the test, which in itself

can cause reflex lacrimation in some people and thus affect the accuracy of the test [5].

Another indicator for tear film function is the patient blinking rate per minute which was found to correlate positively to the severity of subjective dry eye symptoms. Dry eye subjects were found to have significantly higher blinking rates. Reduced and incomplete blinking along with increased tear film break-up during normal visual tasks may explain the increased level of ocular discomfort symptoms reported at the end of the day, particularly in dry eye patients. The normal average resting blinking rate per minute is 17, increasing to around 26/min during conversation and decreasing to around 4.5/min while reading [6, 7].

Tear film function and stability in diabetic patients are an area of ongoing active research. To our knowledge, a few studies have investigated the correlation between the quantity and quality of tears [8, 9] but a few or almost no studies examined this correlation in diabetic patients and compared them to age matched controls.

The purpose of this study is to study the tear film quantity (represented by AS OCT measured inferior tear meniscus height) and correlate it with the quality (represented by the TTT for stability, and patient blinking rate per minute) in diabetic patients and age matched controls.

2. Subjects and Methods

The study was done in accordance with the ethical standards in the Declaration of Helsinki 1964 [10]. An informed consent was taken from all patients before participating in the study.

In this case control prospective series, 22 diabetic patients (9 males and 13 females), that is, Group A, and 16 age matched controls (7 males and 9 females), that is, Group B, were recruited from Kasr Al Aini (Cairo University Hospital) Ophthalmology and Diabetology out-patient clinics.

The age of the patients ranged from 40 to 70 years (mean 57.22 ± 8.5 SD) for the diabetic patients (Group A) and from 30 to 77 years (mean 57.12 ± 12.22 SD) for the nondiabetic group (Group B).

A full history was taken, including detailed ocular and medical history, as well as a thorough full ophthalmological examination. A routine laboratory work-up was also done for all patients.

The inclusion criteria for patients were as follows:

(i) A history of diabetes mellitus for a duration ≥5 years for cases in Group A and no history of any systemic disease for group B.

(ii) No history of chronic eye diseases and/or previous ocular surgery.

(iii) No symptoms of dry eye.

(iv) No history of associated systemic conditions.

(v) No use of systemic or topical medications that may affect the tear film secretion like parasympathomimetics or parasympatholytics.

The patient blinking rate per minute was measured in all patients, by the same observer who attended during all patient examinations and was asked to count the rate of blinking while the patient was having a routine conversation with his doctor.

For assessment of tear film quantity the tear meniscus height (TMH) was measured by the spectral domain optical coherence tomography (RTVue100-2, Optovue, CA, USA) with the cornea-anterior segment lens long attached. Each patient was placed at the chin rest of the AS OCT machine. The tear meniscus height (TMH) was measured through applying a vertical line scan passing the cornea, the inferior tear meniscus, and the lower eyelid at the 6 o'clock position. In the resulting triangular image of the inferior tear meniscus, the height of the limb opposite the acute angle formed between the inferior cornea and the lower lid margin was measured manually using the machine calipers and expressed in microns.

For the assessment of tear film stability, a subtype of the noninvasive BUT, the tear thinning time (TTT), was performed for all cases. Each subject was instructed to place their chin on the chin rest of the keratometer (CIOM SRL, Milano, Italy). The patient was then asked to blink several times and then open his eyes and stop blinking. The observer then counted the time elapsed in seconds between the last blink and the earliest change in shape of the keratometer's mires reflected upon the patient's corneal surface. The time was then recorded in seconds. The tear thinning time is usually normally shorter than the TBUT, as tear film thinning happens seconds before actual tear break-up.

For each measured parameter, three successive readings were taken for each patient, and the average was taken for each case.

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann-Whitney U test for independent samples. For comparing gender, Chi square (χ^2) test was performed. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for nonnormal variables/nonlinear monotonic relation. A P value less than 0.05 was considered statistically significant. All statistical calculations were done using the computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA).

3. Results

This is a nonrandomized controlled case study, where twenty-two diabetic patients (Group A), attending the out-patient clinic for routine checkup, who met the inclusion criteria for the current study were recruited.

The obtained data under study were compared to those obtained from a control group of nondiabetic patients (Group B), who still met the inclusion criteria for the current work.

The demographic data of patients enrolled in the study is summarized in Table 1.

The duration of diabetes in group A patients ranged from 5 to 30 years duration with a mean duration of 13.5 ± 7.5 (SD).

Table 1: Demographic data of patients.

	Group A	Group B	P value
Age	40–70 yrs (57.27 ± 8.5)	30–77 yrs (57.12 ± 12.22)	0.871
Females	13 (59.1%)	9 (56.2%)	0.861
Males	9 (40.9%)	7 (43.8%)	

3.1. The Tear Thinning Time (TTT). TTT was found to range from 9 to 22 seconds with a mean value of 14.36 sec ± 3.38 in the diabetic patients (Group A), as compared to a mean value of 14.75 sec ± 3.43 in the control group (Group B); the difference between the two groups was found to be statistically insignificant ($P = 0.6$).

Moreover, the recorded mean value was 13.85 sec ± 2.99 in the diabetic females in Group A, as compared with a mean value of 15.11 sec ± 3.95 in the diabetic males within the same group; the difference was noted to be of no statistical significance ($P = 0.5$).

3.2. The Blinking Rate. The rate of blinking per minute was recorded to vary from 18 to 40 blinks/min with a mean value of 25.32/min (±6.15 SD) in Group A patients as compared to a range from 15 to 24 blinks/min with a mean of 20.44/min (±2.25 SD) in group B; the difference between the two groups was found to be statistically highly significant ($P = 0.002$).

Moreover, the rate of blinking was found to be lower among diabetic females when compared to diabetic males in Group A, with mean rates of 22.85/min (±3.75) and 28.89/min (±7.35), respectively. This difference was statistically significant ($P = 0.02$).

3.3. The Tear Meniscus Height at the Centre (TMH). The TMH ranged from 163 to 740 μm with a mean of (365.64 ± 148.6 SD) in Group A patients as compared to a range from 171 to 727 μm (356.38 ± 155.75 SD) in Group B patients, with no significant statistical difference ($P = 0.96$).

Regarding the sex differences, the TMH was found to be less in diabetic males as compared to diabetic females in Group A with a mean value of 349.56 μm ± 140.07 and 376.77 μm ± 158.89, respectively, although the difference was statistically insignificant ($P = 0.81$).

3.4. The Correlation between the Studied Variables in Group A. Generally, a negative correlation of varying strength was found between the age of the patient and the three studied variables (the TMH, TTT, and blinking rate/minute) as shown in (Table 2).

A strong negative correlation ($r = -0.625$) was found between the age of patient and the TMH in microns, a correlation that was found to be statistically highly significant ($P = 0.002$).

The weak negative correlation between the age and the rate of blinking ($r = -0.39$) was of no statistical significance ($P = 0.06$); however it was close to being significant. The weak negative correlation between age and TTT was of no statistical significance ($P = 0.67$).

Upon correlating the three tear film parameters with each other, a weak positive correlation ($r = 0.23$) was recorded between the TTT and blinking rate/min (representing stability) and the TMH in microns (representing quantity), although it was statistically not significant ($P = 0.28$).

The rate of blinking was found to be positively correlated to the TTT, again with no statistical significance ($P = 0.83$).

A negative correlation was elicited between the studied tear film parameters and the duration of diabetes. This correlation was of statistical significance regarding the blinking rate and TMH ($P = 0.03$), although it failed to achieve a statistical significance regarding TTT ($P = 0.35$).

A summary of the correlation between the different studied parameters in Group A is elaborated in Table 2.

3.5. The Correlation between the Studied Variables in Group B. A weak negative correlation between the age of the patient and TTT as well as TMH was elicited (Table 3) that was noted to be statistically nonsignificant. However, a weak positive correlation between the rate of blinking and age was noted as well, again with no statistical value.

Moreover, a weak negative correlation was recorded between TTT and blinking rate/min ($r = -0.21$) that was considered to be of no statistical significance ($P = 0.4$) as compared to the moderate positive correlation between TTT and TMH ($r = 0.46$) that was noted to be statistically close to being significant ($P = 0.069$).

The rate of blinking was found to be negatively correlated to the TMH, again with no statistical significance ($P = 0.14$).

A summary of the correlation between the different studied parameters in Group B is elaborated in Table 3.

4. Discussion

Diabetes mellitus is a systemic disease that affects mainly the microcirculation and can affect the ocular surface integrity through different mechanisms [11].

The prevalence of dry eye in diabetic patients has been reported to be about 50% in type 2 diabetes. 7% of children with type 1 diabetes were reported to have dry eye manifestations compared to 0% of age matched control children [2].

In the present study, we tried to study the impact of diabetes on the tear film quality and quantity in diabetic patients with no subjective symptoms of dry eye.

We also tried to find a correlation between the different studied tear film parameters in diabetics versus normal controls.

In the present study, the mean TTT was found to be less in the diabetic group as compared to the control group, although not reaching a statistically significant value; this finding agrees with multiple previous studies, which concluded that diabetes can affect the tear film stability [9, 12, 13].

The reason why we preferred to perform the TTT over the regular tear break-up test (TBUT) with fluorescein is the postulated limitations, including the need to instill fluorescein dye, lack of standardization of fluorescein concentration or amount, and the possible induction of reflex tearing that might alter the results of the test [5].

TMH is typically used to detect the amount of the aqueous layer of the tear film. In our study it was noted to have a mean value higher in the diabetic than in the control

Table 2: The cross relations in Group A.

		Age	TTT	Blinks/min	TMH	DM duration
Age	Correlation coefficient		−0.096	−0.396	−0.625	0.470
	P		0.670	**0.068**	*0.002*	*0.027*
TTT	Correlation coefficient	−0.096		0.047	0.238	−0.208
	P	0.670		0.836	0.286	0.353
Blinks/min	Correlation coefficient	−0.396	0.047		0.239	−0.446
	P	**0.068**	0.836		0.285	*0.037*
TMH	Correlation coefficient	−0.625	0.238	0.239		−0.462
	P	*0.002*	0.286	0.285		*0.030*
DM duration	Correlation coefficient	0.470	−0.208	−0.446	−0.462	
	P	*0.027*	0.353	*0.037*	*0.030*	

Table 3: The cross relations in Group B.

		Age	TTT	Blinks/min	TMH
Age	Correlation		−0.155	0.025	−0.028
	P		0.567	0.928	0.917
TTT	Correlation	−0.155		−0.218	0.466
	P	0.567		0.418	0.069
Blinks/min	Correlation	0.025	−0.218		−0.383
	P	0.928	0.418		0.143
TMH	Correlation	−0.028	0.466	−0.383	
	P	0.917	**0.069**	0.143	

group, yet with no statistical significance. Again, this agreed with previous published results that diabetic patients might show a decrease in BUT, a decrease in basal secretion yet with a normal overall tear secretion [9].

Diabetes is one of the well-established causes of excessive blinking (blinking eye syndrome) [11]. However, some authors reported a decrease in the blinking rate in diabetics but with an increase in the interblinking interval [14].

In our study, the rate of blinking per minute was found to be significantly higher in the diabetic as compared to the control group. We owe this to the changes in ocular surface integrity (namely, recurrent epithelial defects) associated with diabetes with subsequent irritation. Furthermore, blinking was found to be significantly higher in females as compared to males within the diabetic group. This gender difference in blinking rate needs to be further investigated in more detail, as other hormonal factors may be involved.

Several studies demonstrated that dry eye could be correlated with the duration of diabetes as well as the severity of retinopathy [15, 16]; however, others postulated that the metabolic control might be of great impact rather than the duration [2, 8].

In our study, we did not study the correlation with the severity of the disease; however, a statistically significant impact could be elicited regarding the effect of duration of diabetes on tear production (i.e., TMH) as well as on the rate of blinking and the TTT. All values showed further decrease with increasing duration of diabetes.

The absence of subjective symptoms of dryness (as all our patients did not complain of dry eye symptoms) despite the low values of TTT in the diabetic group can be explained by the fact that the associated decrease in corneal sensitivity caused by diabetic peripheral neuropathy in those patients might mask the symptoms of dryness.

Previous studies demonstrated that the impact of diabetes on ocular surface integrity and subsequently on dry eye associated with the disease might be affected with the decreased circulating sex hormones in the postmenopausal diabetic females, a thing that might add to the severity of the condition [11]. This point was not investigated in depth in our work. However, we noted that the TTT values were found to be less in diabetic females as compared to males, despite higher TMH values. Whether this is or is not related to menopause or is merely a gender difference needs to be further investigated in detail in subsequent studies.

The mechanisms by which diabetes affects the integrity of the ocular surface, and the tear film stability and tear production have been previously investigated, whereas the impact of diabetes might also be related to the microcirculation of the lacrimal gland, a point which needs to be further correlated with tear film parameters [17, 18].

Some studies suggested that the decreased corneal sensitivity and corneal neuropathy to be a cause of decreased basal tear secretions in diabetics [16]. Moreover, an inflammatory theory, with the postulation that dry eyes might be secondary to an inflammatory process mediated through T-call lymphocytes was also suggested by some authors [9].

In conclusion, our study showed that the tear film integrity was found to be affected in diabetic patients, as compared to age matched controls, especially when it comes to the blinking rate. A significant negative correlation was found between the duration of diabetes and all studied tear film parameters. The correlation between the volume and stability of the tear film in diabetics was found to be a positive one. A significant difference in blinking rate was found between males and females in the diabetic group, the exact cause of which needs further investigation.

The associated neuropathy with the secondary decrease in corneal sensitivity might add to the severity of the condition, yet with minimal symptoms. Our study thus recommends that a routine examination for dry eye should be considered

in all diabetic patients even in the absence of subjective symptoms.

In diabetics the tear film stability might be affected, despite an increase in tear production that is, TMH, due to the fact that diabetes can affect the basal tear production mainly, rather than the total tear production. This again should not trick us into excluding dry eye in a diabetic patient depending only on the normal TMH measurements [9].

The mechanism of dry eye in diabetes is a multifactorial one, where the diagnosis should be based on the measurement of multiple tear parameters rather than a single one, as not all the parameters are affected in a similar pattern.

The severity of the condition might be related to the severity of diabetes and the metabolic control of the disease; however, the duration of the disease plays a significant role as well.

Conflict of Interests

The authors have no financial interest in any of the products mentioned in this study.

References

[1] C. S. de Paiva, J. L. Lindsey, and S. C. Pfiugfelder, "Assessing the severity of keratitis sicca with videokeratoscopic indices," *Ophthalmology*, vol. 110, no. 6, pp. 1102–1109, 2003.

[2] L. Yu, X. Chen, G. Qin, H. Xie, and P. Lv, "Tear film function in type 2 diabetic patients with retinopathy," *Ophthalmologica*, vol. 222, no. 4, pp. 284–291, 2008.

[3] K.-C. Yoon, S.-K. Im, and M.-S. Seo, "Changes of tear film and ocular surface in diabetes mellitus," *Korean Journal of Ophthalmology*, vol. 18, no. 2, pp. 168–174, 2004.

[4] S. L. Misra, J. P. Craig, D. V. Patel et al., "In vivo confocal microscopy of corneal nerves: an ocular biomarker for peripheral and cardiac autonomic neuropathy in type 1 diabetes mellitus," *Investigative Ophthalmology & Visual Science*, vol. 56, no. 9, pp. 5060–5065, 2015.

[5] T. Kojima, R. Ishida, M. Dogru et al., "A new noninvasive tear stability analysis system for the assessment of dry eyes," *Investigative Ophthalmology and Visual Science*, vol. 45, no. 5, pp. 1369–1374, 2004.

[6] A. R. Bentivoglio, S. B. Bressman, E. Cassetta, D. Carretta, P. Tonali, and A. Albanese, "Analysis of blink rate patterns in normal subjects," *Movement Disorders*, vol. 12, no. 6, pp. 1028–1034, 1997.

[7] N. L. Himebaugh, C. G. Begley, A. Bradley, and J. A. Wilkinson, "Blinking and tear break-up during four visual tasks," *Optometry and Vision Science*, vol. 86, no. 2, pp. E106–E114, 2009.

[8] M. R. Manaviat, M. Rashidi, M. Afkhami-Ardekani, and M. R. Shoja, "Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients," *BMC Ophthalmology*, vol. 8, article 10, 4 pages, 2008.

[9] H. Lv, A. Li, X. Zhang et al., "Meta-analysis and review on the changes of tear function and corneal sensitivity in diabetic patients," *Acta Ophthalmologica*, vol. 92, no. 2, pp. e96–e104, 2014.

[10] World Medical Association Declaration of Helsinki, "Ethical principles for medical research involving human subjects," *Journal of the American Medical Association*, vol. 310, no. 20, pp. 2191–2194, 2013.

[11] S. L. Misra, D. V. Patel, C. N. McGhee et al., "Peripheral neuropathy and tear film dysfunction in type 1 diabetes mellitus," *Journal of Diabetes Research*, vol. 2014, Article ID 848659, 6 pages, 2014.

[12] M. Dogru, B. Kaderli, O. Gelisken et al., "Ocular surface changes with applanation contact lens and coupling fluid use after argon laser photocoagulation in non insulin dependent diabetes mellitus," *American Journal of Ophthalmology*, vol. 138, no. 3, pp. 381–388, 2004.

[13] L. C. Figueroa-Ortiz, E. Jiménez Rodríguez, A. García-Ben, and J. García-Campos, "Study of tear function and the conjunctival surface in diabetic patients," *Archivos de la Sociedad Española de Oftalmología*, vol. 86, no. 4, pp. 107–112, 2011.

[14] K. Inoue, K. Okugawa, S. Amano et al., "Blinking and superficial punctate keratopathy in patients with diabetes mellitus," *Eye*, vol. 19, no. 4, pp. 418–421, 2005.

[15] M. Ozdemir, M. A. Buyukbese, A. Cetinkaya, and G. Ozdemir, "Risk factors for ocular surface disorders in patients with diabetes mellitus," *Diabetes Research and Clinical Practice*, vol. 59, no. 3, pp. 195–199, 2003.

[16] J. Saito, M. Enoki, M. Hara, N. Morishige, T.-I. Chikama, and T. Nishida, "Correlation of corneal sensation, but not of basal or reflex tear secretion, with the stage of diabetic retinopathy," *Cornea*, vol. 22, no. 1, pp. 15–18, 2003.

[17] R. I. Fox, "Sjogren's syndrome," *Current Opinion in Rheumatology*, vol. 7, pp. 409–416, 1995.

[18] X. J. Song, D.-Q. Li, W. Farley et al., "Neurturin-deficient mice develop dry eye and keratoconjunctivitis sicca," *Investigative Ophthalmology & Visual Science*, vol. 44, no. 10, pp. 4223–4229, 2003.

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Clinical Study

Effectiveness and Optical Quality of Topical 3.0% Diquafosol versus 0.05% Cyclosporine A in Dry Eye Patients following Cataract Surgery

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Purpose. To evaluate the effectiveness and optical quality of 3.0% topical diquafosol versus 0.05% cyclosporine A in dry eye patients following cataract surgery. *Methods.* In total, 40 eyes of 40 patients newly diagnosed with dry eye syndrome 1 week after cataract surgery were randomized to receive either 3.0% diquafosol ophthalmic solution six times daily or 0.05% cyclosporine A twice daily for 3 months. Outcome measures were tear film break-up time (TBUT), results on Schirmer 1 test, ocular surface staining score, the ocular surface disease index (OSDI) score, and higher-order aberrations (HOAs). Measurements were taken at baseline and at 1, 2, and 3 months. *Results.* In the diquafosol group, TBUT showed higher outcomes than the cyclosporine A group at 1 and 3 months. Both groups showed increased scores on Schirmer 1 test. The ocular surface staining score decreased in all periods in both groups. Vertical coma and total HOAs decreased more in the cyclosporine A group than in the diquafosol group at 3 months. *Conclusion.* Both 3.0% diquafosol and 0.05% cyclosporine A were effective in treating dry eye after cataract surgery. Diquafosol was more effective in increasing the tear secretion, but cyclosporine A was more effective in improving optical aberrations.

1. Introduction

Cataract surgery has undergone major advances in treating visual loss in cataract patients. For example, the smaller incision site of phacoemulsification decreases postoperative complications and shortens recovery time. It is currently assumed that the use of advanced technology in cataract surgery decreases postoperative symptoms. However, many patients still complain of irritation, blurring, and visual disturbances after surgery. A possible reason for this is dry eye caused by tear film instability after surgery [1, 2]. Dry eye not only affects the ocular surface, leading to irritable symptoms but also affects vision and overall quality of life. There are numerous causes of dry eye, including nerve cell injury during surgery, ocular epithelial injury due to corneal exposure, and use of conventional postoperative eye drops such as anti-inflammatory agents, topical corticosteroids, and anti-infectives [2–4].

Tear film instability during dry eye is the most common cause of decreased optical qualities of the eye. Previous studies suggested that this decrease is the primary cause of blurry vision associated with dry eye syndrome and tear film disruption [5]. Tear film changes in dry eyes may lead to irregularities of the corneal surface [6], and previous studies have reported that dry eyes can have an irregular tear film distribution across the cornea [7, 8].

To treat dry eye syndrome, numerous pharmacological eye drops have been used, including artificial tears, corticosteroids, autologous serum, sodium hyaluronate, and immunomodulators. As a commonly used eye drop, 0.05% cyclosporine A is a potent immunomodulatory agent that inhibits T-cell activation and downregulates the production of inflammatory cytokines, resulting in reduced surface inflammation [9–11]. Three percent diquafosol is a P2Y2 purinergic receptor agonist that activates P2Y2 receptors on the ocular surface

[12]. Diquafosol stimulates both fluid secretion from the conjunctival epithelial cells and mucin secretion from the conjunctival goblet cells directly on the ocular surface, by interacting with the P2Y2 receptors to increase the tear film stability [12].

Previous studies have described the mechanisms and outcomes of these two topical eye drops [9, 11–14]. They differ in their mechanisms of action on dry eye, possibly leading to different outcomes. No previous study has compared and standardized the effects and optical quality of these two types of eye drops, but this would be valuable to optimize the treatment of dry eye patients after cataract surgery. Therefore, the objective of the current study was to compare the efficacy and safety of these two types of topical eye drops in stabilizing the tear film to treat dry eye patients following cataract surgery.

2. Material and Methods

2.1. Study Design. This study was a prospective, open-label, randomized, controlled study that followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board at Kangdong Sacred Heart Hospital (agreement number 2015-04-002-006). After receiving an explanation of the nature and possible consequences of the study, all patients provided informed consent before being treated.

2.2. Patients. In total, 45 eyes of 45 patients newly diagnosed with dry eye syndrome 1 week after cataract surgery were enrolled in the study. They had undergone phacoemulsification and an intraocular lens implantation between April 2015 and June 2015. All surgeries were performed by a single surgeon. A monofocal aspheric, hydrophilic, acrylic intraocular lens with aberration neutrality (Akreos ADAPT AO, Baush & LOMB, USA) was implanted using an injector following ultrasonic emulsification. Subjects were aimed for emmetropia using an appropriate intraocular lens as measured by the SRK/T formula. None of the subjects were aimed for monovision in this study. After the operation, patients used topical antibiotics (Vigamox®, moxifloxacin hydrochloride; Alcon, Fort Worth, TX, USA) and a topical steroid (Pred Forte®, 1% prednisolone acetate; Allergan, Dublin, Ireland) four times a day for 5 weeks.

At 1 week after cataract surgery, only patients with mild to moderate dry eye syndrome were included in this study, involving an Oxford score of 1–3 and a TBUT of 3–9 seconds regarding the 2007 International Dry Eye Workshop criteria [15]. Patients with severe dry eye syndrome (Oxford score $\geq$ 4, and a TBUT $\leq$ 2 seconds), other diseases affecting tear film stability, or continuous dry eye medication previous to cataract surgery were excluded.

2.3. Randomization and Treatment Administration. Patients who provided informed consent were enrolled in the study by their treating physician and were assigned a sequential number with a corresponding randomization code generated by an independent third party using SAS software (version 8.0, SAS Institute Inc., Cary, NC). According to the randomization code, clinical staff assigned patients to receive either 3.0% diquafosol ophthalmic solution six times daily or 0.05% cyclosporine A twice daily from 1 week to 3 months after cataract surgery. The clinical staff provided treatment medications and instructions on how to administer ophthalmic solutions according to the assigned randomization group.

2.4. Outcome Measures. Patient's baseline characteristics were measured at 1 week after cataract surgery by an ophthalmological examination, with additional evaluation of ocular surface fluorescein staining (grades 0–5, according to the Oxford score), tear break-up time (TBUT), Schirmer 1 test results, and optical aberrations using a Hartmann-Shack wavefront aberrometer (WASCA; Carl Zeiss Meditec, Oberkochen, Germany). Optical aberrations were recorded in mesopic conditions without any pharmacologic mydriasis, were analyzed by expanding the set of Zernike polynomials, and were expressed for the central 4 mm diameter [16, 17]. Patients were reexamined at 1, 2, and 3 months after surgery, and the ocular surface disease index (OSDI) questionnaire was surveyed at each visit.

2.5. Statistical Analysis. SPSS for Windows software (ver. 18.0; SPSS, Chicago, IL, USA) was used for statistical analyses. Categorical data were analyzed using the Fisher's exact test. The changes in continuous variables from baseline were analyzed using the Wilcoxon signed rank test. The Mann-Whitney U test was used to compare changes in continuous variables between the two groups. A P value <0.05 was considered statistically significant.

3. Results

Forty eyes qualified and completed all study procedures. No intraoperative complications occurred in any of the surgeries (e.g., posterior capsular tear and vitreous loss). There was no patient presenting corneal edema at 1 week after surgery in both groups. The mean age was 64.3 ± 9.44 years in the diquafosol group (n = 20) and 63.4 ± 12.2 years in the cyclosporine A group (n = 20). Baseline clinical characteristics showed no differences in age, sex, spherical equivalent, OSDI, TBUT, Schirmer 1 test results, ocular surface staining scores, or ocular aberrations (Table 1).

TBUT showed improvement at 1, 2, and 3 months in the diquafosol group, but only at the second month in the cyclosporine A group (Figure 1). The diquafosol group showed better TBUT results at 1 month ($P < 0.001$) and 3 months ($P = 0.001$) when compared with the cyclosporine A group (Figure 1). Schirmer 1 test scores increased at 2 months ($P < 0.05$) and 3 months ($P < 0.001$) in the diquafosol group, and at 1 month ($P < 0.05$) and 3 months ($P = 0.006$) in the cyclosporine A group (Figure 2). The diquafosol group had higher scores on Schirmer 1 test at 3 months, although the group difference was not statistically significant ($P = 0.06$). Ocular surface staining showed improvement at all periods for both groups, but there was no significant difference between the two groups (Figure 3). All OSDI scores (e.g., symptom intensity, frequency, and aggravation) showed

Table 1: Baseline characteristics in the diquafosol and cyclosporin group.

	Diquafosol ($n = 20$)	Cyclosporin A ($n = 20$)	P
Age (yrs)	64.3 ± 9.44	63.4 ± 12.2	0.892*
Gender ratio (M/F)	0.67	0.67	0.750†
Spherical equivalent (D)	−0.75 ± 0.45	−0.5 ± 0.65	0.368*
OSDI index (0–100)	20.1 ± 13.88	22.29 ± 13.66	0.798*
TBUT (s)	3.17 ± 1.01	3.7 ± 1.08	0.141*
Schirmer 1 test (mm/5 min)	7.7 ± 3.68	8.4 ± 3.60	0.278*
Oxford score (0–5)	1.41 ± 0.62	1.5 ± 0.83	0.798*
Total aberration (μm)	1.00 ± 0.58	0.87 ± 0.35	0.684*
Total HOAs (μm)	0.33 ± 0.22	0.27 ± 0.10	0.752*
Vertical coma (μm)	0.20 ± 0.19	0.27 ± 0.22	0.357*
Horizontal coma (μm)	0.28 ± 0.21	0.32 ± 0.29	0.916*
Vertical trefoil (μm)	0.25 ± 0.19	0.23 ± 0.24	0.478*
Oblique trefoil (μm)	0.40 ± 0.34	0.29 ± 0.22	0.442*
Spherical aberration (μm)	0.20 ± 0.13	0.21 ± 0.14	0.707*

D = diopter; M = male; F = female; OSDI = ocular surface disease index; TBUT = tear break-up time; HOAs = higher-order aberrations.
Mean ± standard deviation.
There was no statistically significant difference between 2 groups in baseline characteristics by *Mann-Whitney U test or †Fisher's exact test ($P > 0.05$).

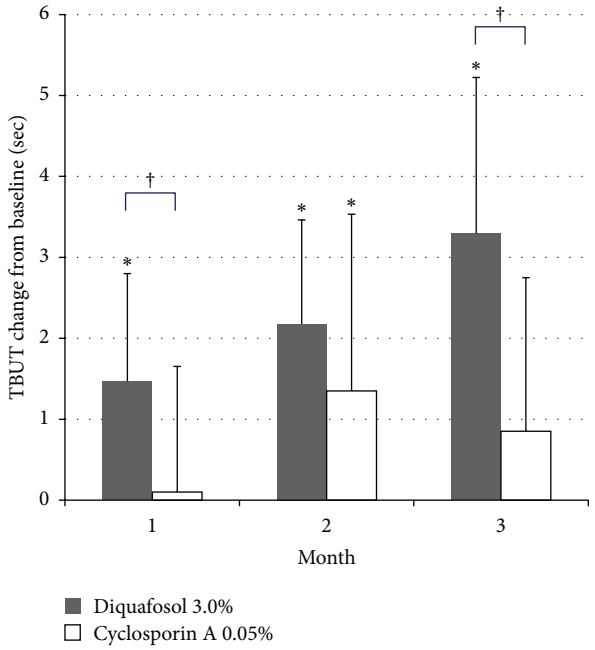

Figure 1: Change in tear break-up time (TBUT) from baseline. Mean value + standard deviation. ∗ = statistically significant difference in changes of TBUT from baseline ($P < 0.05$, Wilcoxon signed rank test). † = statistically significant difference in TBUT between the two groups ($P < 0.05$, Mann-Whitney U test).

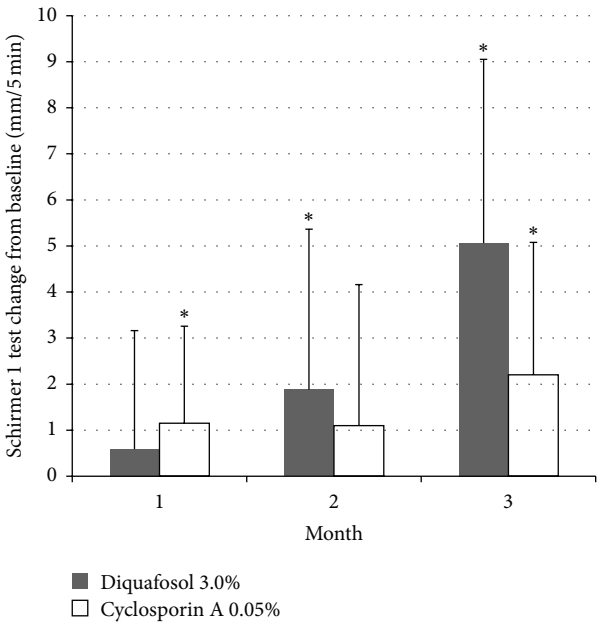

Figure 2: Change in Schirmer 1 test score from baseline. Mean value + standard deviation. ∗ = statistically significant difference in changes of Schirmer 1 test from baseline ($P < 0.05$, Wilcoxon signed rank test).

a decreasing pattern throughout the treatment period in both groups and failed to show a significant improvement (Figure 4). Total HOAs showed improvement in the cyclosporine A group at 2 months ($P < 0.05$) and 3 months ($P = 0.002$) and better results compared with the diquafosol group at 3 months ($P < 0.05$) (Figure 5(a)). Vertical coma showed an improvement in the cyclosporine A group at 3 months ($P < 0.05$) and a significant difference compared with the diquafosol group at 2 months ($P < 0.01$) and 3 months ($P < 0.05$) (Figure 5(b)). All other optical aberration values showed no significant changes.

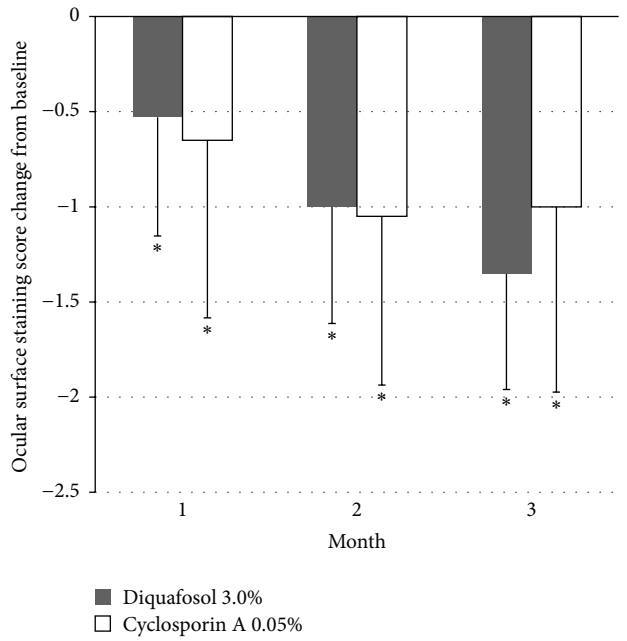

Figure 3: Change in ocular surface staining score from baseline. Mean value − standard deviation. ∗ = statistically significant difference in changes of ocular surface staining score from baseline ($P < 0.05$, Wilcoxon signed rank test).

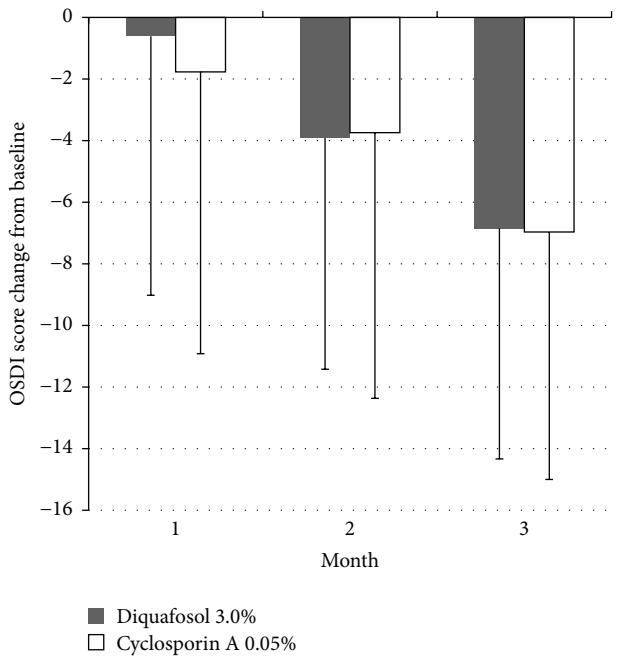

Figure 4: Change in the ocular surface disease index (OSDI) score from baseline. Mean value − standard deviation. All OSDI scores showed a decreasing pattern throughout the treatment period in both groups, but there is no statistically significant difference.

4. Discussion

Dry eye syndrome is the most common disorder of the eye, and numerous theories of its pathogenesis, as well as numerous treatment options, have been reported. Dry eye after cataract surgery develops as a result of damage to the long ciliary nerves. Nerve injury leads to a decrease in blinking and tear production, resulting in permeability and metabolic abnormalities that result in dry eye [18, 19]. In addition, corneal incisions made during cataract surgery can release inflammatory mediators that reduce corneal sensitivity and tear film stability [15, 20]. Consistent with this theory, a previous study reported that OSDI, TBUT, and tear secretion decrease after cataract surgery [20]. Various treatments, especially cyclosporine A, have shown improvement of dry eye after cataract surgery. Cyclosporine A affects cytokine levels to control inflammation and has been shown to be an effective dry eye treatment, as assessed by Schirmer 1 test, the TBUT, and the OSDI [13]. The mechanism of action of cyclosporine A in dry eye is not completely known, but one possible mechanism involves the inhibition of cytokine production by activated T lymphocytes, resulting in reduced inflammation of the ocular surface and improved tear film stability [21, 22]. Consistent with this possibility, Jeon et al. reported that treatment with 0.05% cyclosporine A led to tear film stability. In this study, tear film instability was caused by inflammation due to corneal incisions, so controlling the inflammation led to improved stability [23]. Inflammation may therefore be a significant factor in tear film instability, leading to increased optical aberrations. There were some reports that improvement of tear film stability led to improvement in optical aberrations [17, 24]. In our study, 0.05% cyclosporine A treatment resulted in a significant decrease in optical aberrations due to a decrease of inflammation. Furthermore, 0.05% cyclosporine A treatment showed improvement at 2 and 3 months postoperatively, suggesting that improvement of optical aberrations by inflammatory modulators such as 0.05% cyclosporine A requires a relatively long period of continuous medication. In contrast, 3.0% diquafosol showed a rapid increase in TBUT and Schirmer 1 test results, which resulted in OSDI scores that reflected a decrease in dry eye symptoms.

The P2Y2 receptor agonist activates calcium secretion in the conjunctival epithelial and endoplasmic reticulum cells to increase secretion of mucin. Mucin performs an important role in protecting the surface of the cornea and stabilizing the tear film [25, 26]. In the current study, stabilized tear film led to increased TBUT and improvement in the Oxford scheme test grades. Furthermore, patients in our study showed rapid improvement of ocular surface dryness, leading to a decrease in dry eye symptoms. Although there was improvement in the TBUT and corneal staining, 3.0% diquafosol showed no significant decrease in optical aberrations. Meanwhile, there was no significant improvement in OSDI scores. Because the patients included in the study were limited to those with mild to moderate dry eye, it is possible that the improvement of symptoms did not significantly affect the scores.

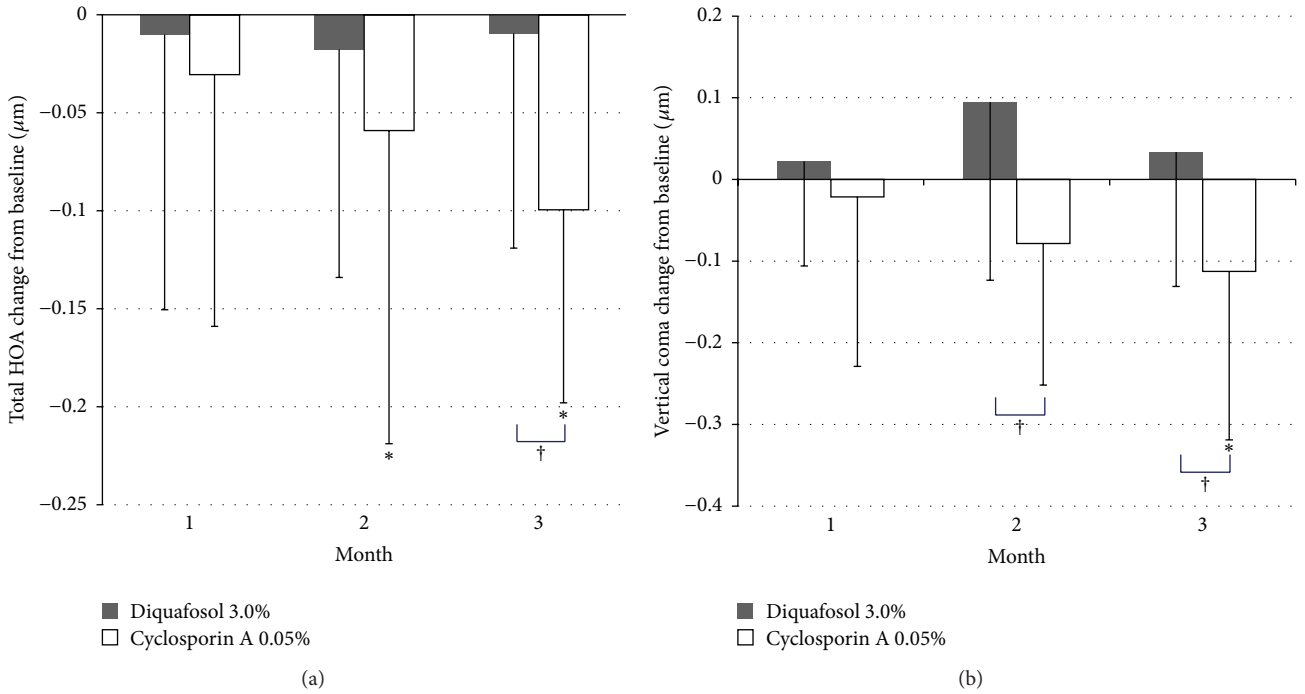

Figure 5: (a) Change in total higher-order aberrations (HOAs) from baseline. (b) Change in vertical coma from baseline. Mean value − standard deviation. ∗ = statistically significant difference in changes of aberrations from baseline ($P < 0.05$, Wilcoxon signed rank test). † = statistically significant difference in aberrations between the two groups ($P < 0.05$, Mann-Whitney U test).

Treatment with 3.0% diquafosol causes excessive secretion of fluid that paradoxically disturbs tear film stability. Choi and Shin reported that excessive secretion of tears (>10 mm) affected HOAs more than moderate secretion (6–10 mm) of tears [24]. The appropriate amounts of mucin and fluid stabilize the tear film, but excessive secretion due to continuous medication destabilizes this film. In addition, the P2Y2 receptor can play a role in the control of inflammation, so immediate treatment with 3.0% diquafosol after cataract surgery can affect inflammation [27]. In this study, the patients started using 3.0% diquafosol and 0.05% cyclosporine A 1 week after surgery, while using steroid eye drops for control of inflammation. Hyperemia, discharge, and anterior chamber reaction, which are regarded as symptoms of inflammation, were not observed in patients treated with 3.0% diquafosol. Also, there were no adverse effects in patients treated with 0.05% cyclosporine A.

There are some limitations to the present study. First, we enrolled patients with mild to moderate dry eye that met the 2007 International Dry Eye Workshop criteria. These means that our results should not be applied to all dry eye patients, in particular those requiring more intensive treatment. Also, this study was limited due to a small number of patients and the occasional lack of patient compliance. Five out of 45 patients failed to maintain the prescribed eye drop regimen and were excluded from the study (3 patients in the cyclosporine A group and 2 patients in the diquafosol group). Another factor interfering our study was that our aberrometric values were based on optical aberration. The optical aberrations are influenced by the tear film, the cornea, and the lenticular state. In particular, lenticular change such as posterior capsular opacification may affect the optical aberrations and interfere with the evaluation of the aberrometric values in dry eye. However, there was no patient with posterior capsular opacification throughout the follow-up period in this study. Although we did not observe any complications in all patients during follow-up period, the minimal positional change of IOL and capsule contracture may be correlated with HOAs values. Third, either 0.05% topical cyclosporine A or 3.0% diquafosol was additionally administered to the patients with the topical antibiotics and steroids during the same period from 1 to 5 weeks after surgery. In order to evaluate the effectiveness of the drug accurately, other drugs should not be used simultaneously or clearance period might be needed. However, we had focused on the additive treatment of the patient complaining of the symptoms of dry eye immediately after cataract surgery. Finally, this study is an open-label study using commercial eye drops. That means subjective factors cannot be excluded. The absence of blindness might affect the study results.

5. Conclusion

Both 3.0% diquafosol and 0.05% cyclosporine A were effective for the improvement of objective signs and subjective symptoms of dry eye after cataract surgery, but the timing

and degree of therapeutic effects on tear film and ocular parameters differed between the two medications. Three percent diquafosol was more effective in increasing TBUT and Schirmer 1 test scores, while 0.05% cyclosporine A decreased optical aberrations.

Disclosure

The authors have no proprietary or financial interest in any aspect of this report.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Conception and design were done by Jang Hoon Lee, In Seok Song, Kyoung Lae Kim, and Sam Young Yoon. Data collection was performed by Jang Hoon Lee. Analysis and interpretation of data were carried out by Jang Hoon Lee, In Seok Song, Kyoung Lae Kim, and Sam Young Yoon. Writing of the paper was done by Jang Hoon Lee and Sam Young Yoon. Critical revision of the paper was carried out by In Seok Song and Sam Young Yoon.

References

[1] Z. Liu, L. Luo, Z. Zhang et al., "Tear film changes after phacoemulsification," *Chinese Journal of Ophthalmology*, vol. 38, no. 5, pp. 274–277, 2002.

[2] K. E. Han, S. C. Yoon, J. M. Ahn et al., "Evaluation of dry eye and meibomian gland dysfunction after cataract surgery," *American Journal of Ophthalmology*, vol. 157, no. 6, pp. 1144.e1–1150.e1, 2014.

[3] N. Kasetsuwan, V. Satitpitakul, T. Changul, and S. Jariyakosol, "Incidence and pattern of dry eye after cataract surgery," *PLoS ONE*, vol. 8, no. 11, Article ID e78657, 2013.

[4] S. Cetinkaya, E. Mestan, N. O. Acir, Y. F. Cetinkaya, Z. Dadaci, and H. I. Yener, "The course of dry eye after phacoemulsification surgery," *BMC Ophthalmology*, vol. 15, article 68, 2015.

[5] R. Montés-Micó, A. Cerviño, T. Ferrer-Blasco, S. García-Lázaro, and D. Madrid-Costa, "The tear film and the optical quality of the eye," *The Ocular Surface*, vol. 8, no. 4, pp. 185–192, 2010.

[6] F.-C. Huang, S.-H. Tseng, M.-H. Shih, and F. K. Chen, "Effect of artificial tears on corneal surface regularity, contrast sensitivity, and glare disability in dry eyes," *Ophthalmology*, vol. 109, no. 10, pp. 1934–1940, 2002.

[7] R. Montés-Micó, A. Cáliz, and J. L. Alió, "Wavefront analysis of higher order aberrations in dry eye patients," *Journal of Refractive Surgery*, vol. 20, no. 3, pp. 243–247, 2004.

[8] R. Montés-Micó, A. Cerviño, T. Ferrer-Blasco, S. García-Lázaro, and S. Ortí-Navarro, "Optical quality after instillation of eye-drops in dry-eye syndrome," *Journal of Cataract and Refractive Surgery*, vol. 36, no. 6, pp. 935–940, 2010.

[9] F. Mah, M. Milner, S. Yiu, E. Donnenfeld, T. M. Conway, and D. A. Hollander, "PERSIST: Physician's Evaluation of Restasis® Satisfaction in Second Trial of topical cyclosporine ophthalmic emulsion 0.05% for dry eye: a retrospective review," *Clinical Ophthalmology*, vol. 6, no. 1, pp. 1971–1976, 2012.

[10] X. Q. Zhou and R. L. Wei, "Topical cyclosporine A in the treatment of dry eye: a systematic review and meta-analysis," *Cornea*, vol. 33, no. 7, pp. 760–767, 2014.

[11] U. Keklikci, B. Dursun, and A. K. Cingu, "Topical cyclosporine a 0.05% eyedrops in the treatment of vernal keratoconjunctivitis—randomized placebo-controlled trial," *Advances in Clinical and Experimental Medicine*, vol. 23, no. 3, pp. 455–461, 2014.

[12] Y. Nagahara, S. Koh, K. Nishida, and H. Watanabe, "Prolonged increase in tear meniscus height by 3% diquafosol ophthalmic solution in eyes with contact lenses," *Clinical Ophthalmology*, vol. 9, pp. 1029–1031, 2015.

[13] Y. W. Chung, T. H. Oh, and S. K. Chung, "The effect of topical cyclosporine 0.05% on dry eye after cataract surgery," *Korean Journal of Ophthalmology*, vol. 27, no. 3, pp. 167–171, 2013.

[14] K. H. Wan, L. J. Chen, and A. L. Young, "Efficacy and safety of topical 0.05% cyclosporine eye drops in the treatment of dry eye syndrome: a systematic review and meta-analysis," *The Ocular Surface*, vol. 13, no. 3, pp. 213–225, 2015.

[15] "The definition and classification of dry eye disease: report of the definition and classification Subcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[16] L. N. Thibos, R. A. Applegate, J. T. Schwiegerling, and R. Webb, "Report from the VSIA taskforce on standards for reporting optical aberrations of the eye," *Journal of Refractive Surgery*, vol. 16, no. 5, pp. S654–S655, 2000.

[17] A. Denoyer, G. Rabut, and C. Baudouin, "Tear film aberration dynamics and vision-related quality of life in patients with dry eye disease," *Ophthalmology*, vol. 119, no. 9, pp. 1811–1818, 2012.

[18] E. D. Donnenfeld, K. Solomon, H. D. Perry et al., "The effect of hinge position on corneal sensation and dry eye after LASIK," *Ophthalmology*, vol. 110, no. 5, pp. 1023–1030, 2003.

[19] M. Kohlhaas, "Corneal sensation after cataract and refractive surgery," *Journal of Cataract and Refractive Surgery*, vol. 24, no. 10, pp. 1399–1409, 1998.

[20] X.-M. Li, L. Hu, J. Hu, and W. Wang, "Investigation of dry eye disease and analysis of the pathogenic factors in patients after cataract surgery," *Cornea*, vol. 26, no. 1, pp. S16–S20, 2007.

[21] S. Matsuda and S. Koyasu, "Mechanisms of action of cyclosporine," *Immunopharmacology*, vol. 47, no. 2-3, pp. 119–125, 2000.

[22] M. E. Stern, J. Gao, T. A. Schwalb et al., "Conjunctival T-cell subpopulations in Sjögren's and non-Sjögren's patients with dry eye," *Investigative Ophthalmology & Visual Science*, vol. 43, no. 8, pp. 2609–2614, 2002.

[23] J. H. Jeon, H. S. Kim, J. W. Jung et al., "Effect of Cyclosporin A on tear film and corneal aberration after cataract surgery," *Journal of the Korean Ophthalmological Society*, vol. 55, no. 7, pp. 978–983, 2014.

[24] S. H. Choi and Y. I. Shin, "Changes in higher order aberration according to tear-film instability analyzed by continuous measurement using wavefront," *Journal of the Korean Ophthalmological Society*, vol. 53, no. 8, pp. 1076–1080, 2012.

[25] H. H. Jung, Y. S. Kang, M. S. Sung, and K. C. Yoon, "Clinical efficacy of topical 3% diquafosol tetrasodium in short tear film break-up time dry eye," *Journal of the Korean Ophthalmological Society*, vol. 56, no. 3, pp. 339–344, 2015.

[26] S. Koh, "Clinical utility of 3% diquafosol ophthalmic solution in the treatment of dry eyes," *Clinical Ophthalmology*, vol. 9, pp. 865–872, 2015.

[27] H. I. Cha, M. S. Jung, D. W. Ahn et al., "Silencing of MUC8 by siRNA increases P2Y(2)-induced airway inflammation," *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 308, no. 6, pp. L495–L502, 2015.

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Research Article

High Levels of 17β-Estradiol Are Associated with Increased Matrix Metalloproteinase-2 and Metalloproteinase-9 Activity in Tears of Postmenopausal Women with Dry Eye

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Purpose. To determine the serum levels of sex steroids and tear matrix metalloproteinases (MMP) 2 and 9 concentrations in postmenopausal women with dry eye. *Methods*. Forty-four postmenopausal women with dry eye and 22 asymptomatic controls were enrolled. Blood was drawn and analyzed for serum levels of sex steroids and lipids. Then, the following tests were performed: tear collection, Ocular Surface Disease Index (OSDI) questionnaire, fluorescein tear film break-up time (TBUT), corneal fluorescein staining, Schirmer test, and conjunctival impression cytology. The conjunctival mRNA expression and tear concentrations of MMP-2 and MMP-9 were measured. *Results*. Serum 17β-estradiol levels were significantly higher in the dry eye subjects than in the controls ($P = 0.03$), whereas there were no significant differences in levels of testosterone, dehydroepiandrosterone sulfate (DHEA-S), and progesterone. Tear MMP-2 and MMP-9 concentrations ($P < 0.001$), as well as the MMP-9 mRNA expression in conjunctival samples ($P = 0.02$), were significantly higher in dry eye subjects than in controls. Serum 17β-estradiol levels were positively correlated with tear MMP-2 and MMP-9 concentrations and negatively correlated with Schirmer test values. *Conclusions*. High levels of 17β-estradiol are associated with increased matrix metalloproteinase-2 and metalloproteinase-9 activity in tears of postmenopausal women with dry eye.

1. Introduction

Epidemiological data have shown that dry eye becomes more frequent with age in both sexes and that women are at a higher risk of dry eye than men [1–3]. The higher prevalence of dry eye in women has been partly attributed to hormonal changes that occur with menstruation, pregnancy, lactation, menopause [4–7], and use of medications such as contraceptives and hormone replacement therapy (HRT) [8]. Sex hormones have been suggested to play a key role in maintaining ocular surface homeostasis.

Ocular surface tissues have been found to be specific targets for sex hormones. Androgen, estrogen, and progesterone receptors have been identified in human lacrimal glands [9], meibomian glands [9, 10], and cornea and conjunctiva [11, 12]. Androgens influence the structure and function of the lacrimal and meibomian glands and exert a significant anti-inflammatory effect on the ocular surface [13, 14]. In contrast, despite the large number of studies, the impact of estrogen and progesterone on the ocular surface tissues is still controversial [15, 16]. Dry eye in postmenopausal women is characterized by both high and low serum estrogen levels and conflicting results [8, 17, 18] have been reported concerning the effect of HRT on the signs and symptoms of dry eye in women. Whether dry eye in females is caused by estrogen excess or deficiency, androgen deficiency or estrogen/androgen relative imbalance remains to be determined.

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that function to maintain and remodel tissue architecture. In addition to their normal roles in tissue remodeling, MMP-2 and MMP-9 are known to be critical extracellular matrix remodeling enzymes in wound healing and diseases of the ocular surface [19]. There are many factors regulating MMP expression, including sex steroids, cytokines, growth factors, and cellular interactions and transformation. Sex steroids such as estrogen and testosterone

have been shown to regulate MMP-2 and MMP-9 expression. Previous studies have shown that estrogen administration increases the expression of MMP-2 and MMP-9 in immortalized human corneal epithelial cells and the lacrimal glands of ovariectomized rabbits or rats [20–22]. It has also been confirmed that MMP activity is upregulated by estrogen in other tissues. For example, estrogen stimulates MMP-2 expression in human granulosa-lutein cells and vascular smooth cells [23] as well as MMP-9 expression in human mesangial cells [24]. Testosterone administration, on the other hand, has been shown to decrease MMP-2 activity in the lacrimal glands of ovariectomized rats [22]. Hence, further studies are required to determine the relationship between sex steroid levels and MMPs in humans.

The aim of this study was to determine the serum sex steroid levels, including 17β-estradiol, testosterone, dehydroepiandrosterone sulfate (DHEA-S), and progesterone, in postmenopausal women with dry eye. Furthermore, we investigated the relationship between sex steroid levels and MMP-2 and MMP-9 activity in tears.

2. Materials and Methods

2.1. Subjects. Between January 2015 and July 2015, dry eye subjects were consecutively recruited from the outpatient clinic and normal control subjects were recruited from a health checkup population at Zhongshan Hospital of Fudan University. This case-control study was approved by the local Ethical Committee and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant before starting the study procedures.

Postmenopausal women aged over 50 years were recruited and later categorized into two groups: the dry eye group and the control group. "Postmenopausal" was defined as no menses for at least 1 year. During a preliminary visit, medical history was assessed, and a comprehensive ophthalmic examination was performed on all participants to ensure eligibility. Dry eye was diagnosed if the subject fulfilled at least two of the following criteria: Ocular Surface Disease Index (OSDI) [25] score > 20, fluorescein tear film break-up time (TBUT) ≤ 5 seconds, and corneal fluorescein staining score > 3 according to the National Eye Institute (NEI) grading scale [26]. The age-matched asymptomatic control subjects exhibited normal results on all of the above measures.

Exclusion criteria for both groups included (1) ceased menses due to autoimmune disorders, smoking, or hysterectomy; (2) a history of Sjogren's syndrome (SS), diabetes, or other systemic disorders known to affect the ocular surface; (3) a history of HRT, contact lens use, or ocular surgery; (4) the use of any topical ocular medication or systemic medication known to exacerbate dry eye; and (5) the presence of anterior segment abnormality or active eye disease other than dry eye.

2.2. Study Protocol. Blood was drawn from each participant by phlebotomists at the Department of Clinical Laboratory, Zhongshan Hospital. Then, tears were collected from the participants, the OSDI questionnaire was administered to the participants, and a series of dry eye tests were performed in the following order: fluorescein TBUT, corneal fluorescein staining, Schirmer test, and conjunctival impression cytology. There was at least a 5-minute gap between each test. Dry eye tests were performed by the same researcher to maintain consistency. Application of artificial tears or other ocular lubricants was discontinued 3 days before each participant's study visit. The temperature and humidity of the examination room were controlled at a range from 20°C to 24°C and from 40% to 50%, respectively. For each subject, the right eye was used for analysis.

2.3. Laboratory Blood Analysis. Blood samples were drawn from all participants at 8:00 a.m. following an overnight fast. Serum levels of sex steroids (17β-estradiol, total testosterone, DHEA-S, and progesterone) were measured using a chemiluminescence method. The limit of detection for the steroids was as follows: 17β-estradiol 5.0 pg/mL (18.35 pmol/L), testosterone 0.087 nmol/L, DHEA-S 0.003 μmol/L, and progesterone 0.095 nmol/L. A serum lipid profile, including total cholesterol, triglycerides, high-density lipoprotein- (HDL-) cholesterol, and low-density lipoprotein- (LDL-) cholesterol, was also obtained as it may influence sex steroid levels [27].

2.4. Tear Sample Collection. Tear samples were collected using disposable 5 μL microcapillary tubes (Microcaps; Drummond Scientific Co., Broomall, PA) without anesthesia. Approximately 5 μL of tear fluids was gathered from the inferior temporal tear meniscus from each eye. Care was taken to ensure that the lid margin, cornea, or conjunctiva was not touched, to avoid as much as possible reflex tears. The tear flow rate was controlled during the process, and only samples with a flow rate of 1–5 μL/min were used for further tests. Tears from both eyes were pooled together and transferred into a 1.5 mL Eppendorf tube and then immediately stored at −80°C until further examination.

2.5. Quantification of MMP-2 and MMP-9 in Tear Samples. Total MMP-2 and MMP-9 (pro- and active forms) concentrations in extracted tear samples were each determined using commercially available quantitative sandwich ELISA kits (Quantikine; R&D Systems, Inc., Minneapolis, MN). Sample preparation and analysis were performed according to the manufacturer's instructions. Tear fluid of precise volume from each sample was transferred and diluted 1 : 20. The final results were corrected according to the dilution factor.

2.6. Assessments of Dry Eye. Dry eye symptoms were assessed using the OSDI questionnaire, which has previously been validated as a reliable method for measuring the severity of dry eye [25]. The OSDI consists of 12 questions about symptoms experienced within the previous week and yields scores ranging from 0 (least severe) to 100 (most severe).

Fluorescein TBUT was measured by instilling 5 μL of 2% sodium fluorescein solution and calculating the time between the last complete blink and the appearance of the first dry spot in the stained tear film. Three consecutive measurements were conducted, and the average value was taken.

Table 1: Demographics and clinical characteristics in patients with dry eye and normal controls.

Characteristics	Dry eye group ($n = 44$)	Control group ($n = 22$)	P value
Age (years)	63.2 ± 7.4	60.7 ± 5.3	0.18
Duration of menopause (years)	11.2 ± 8.0	10.3 ± 5.9	0.81
BMI	22.9 ± 2.2	22.6 ± 2.1	0.70
Lipid profile			
Total cholesterol (mmol/L)	4.5 ± 0.8	4.7 ± 1.0	0.47
Triglycerides (mmol/L)	1.5 ± 0.6	1.6 ± 0.5	0.33
HDL-cholesterol (mmol/L)	1.1 ± 0.3	1.1 ± 0.2	0.36
LDL-cholesterol (mmol/L)	2.8 ± 0.8	3.0 ± 0.9	0.28
OSDI (points)	39.5 ± 24.4	14.0 ± 8.4	<0.001
Fluorescein TBUT (s)	2.5 ± 1.1	9.8 ± 3.9	<0.001
Corneal fluorescein staining (points)	3.1 ± 2.5	0.3 ± 0.4	<0.001
Schirmer test (mm/5 min)	7.1 ± 5.6	12.6 ± 6.2	<0.001

BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein, OSDI = Ocular Surface Disease Index, and TBUT = tear film break-up time.

Corneal fluorescein staining was evaluated under cobalt blue illumination following fluorescein instillation. Corneal staining was assessed using the NEI scale, where grades of 0–3 were assigned for five regions of the corneal surface, up to a total of 15 points.

Then, Schirmer test (without anesthesia) was performed with sterile strips inserted at the border of the medial to the lateral third of the lower lid margin with the lids closed. The moistened length was measured after 5 minutes.

2.7. Conjunctival Impression Cytology. Conjunctival epithelial cells were collected via impression cytology as previously described [28]. Briefly, after administration of topical anesthetic (0.5% proparacaine hydrochloride; Alcon), two sterile membrane filters (6 × 6 mm, Millipore) were gently placed onto the inferotemporal and superotemporal bulbar conjunctiva. Gentle pressure was applied to the filters for 10 seconds using blunt smooth edged forceps. The membranes were then gently removed from the eye and transferred into an Eppendorf tube containing TRIzol reagent (Invitrogen; Carlsbad, CA). The samples were then stored at −80°C until processing.

2.8. Real-Time PCR. Total RNA in conjunctival cell samples was isolated using TRIzol Reagent (Invitrogen) and then reverse-transcribed with Prime-Script RT Master mix (Takara, Otsu, Japan). Gene expression was detected by quantitative real-time PCR using primers for MMP-2, MMP-9, and glyceraldehyde 3-phosphate dehydrogenase (GADPH). The primer sequences used were as follows: MMP-2 (sense: 5′-AGCGAGTGGATGCCGCCTTTAA-3′; antisense: 5′-CATTCCAGGCATCTGCGATGAG-3′); MMP-9 (sense: 5′-GCCACTACTGTGCCTTTGAGTC-3′; antisense: 5′-CCCTCAGAGAATCGCCAGTACT-3′); and GADPH (sense: 5′-GTCTCCTCTGACTTCAACAGCG-3′; antisense: 5′-ACCACCCTGTTGCTGTAGCCAA-3′). Reactions were performed using the Roche LightCycler 480 System (Roche, Indianapolis, IN) in combination with a SYBR Premix Ex Taq Kit (Takara) according to the manufacturer's instructions. The relative gene expression was calculated using the Comparative C_T Method and standardizing levels to GADPH mRNA.

2.9. Data Analysis. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL). Descriptive statistics were presented as the mean ± standard deviation (SD). The sample size (at least 15 eyes at each group) was determined to detect 20% difference in sex steroid levels, with $\alpha = 0.05$ and $\beta = 0.20$. Some values of 17β-estradiol levels were below the limit of assay quantitation (5 pg/mL). Thus, we categorized subjects by 17β-estradiol levels less than 5 pg/mL and by 17β-estradiol levels of 5 pg/mL or greater for analysis. Differences in demographics and measurements between the 2 groups were assessed using Mann-Whitney test for continuous measures and Pearson's chi-squared test for categorical factors. Spearman's rank correlation coefficients between parameters were calculated. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of Subjects. A total of 66 postmenopausal women (66 eyes, mean age 62.4 ± 6.8 years) were enrolled in this study. Among the 66 subjects, 44 subjects were identified as having dry eye based on the diagnostic criteria, and 22 normal control subjects were included for comparison. Baseline demographics and clinical characteristics in patients with dry eye and normal controls are presented in Table 1. No significant differences were noted between the 2 study groups in terms of age, duration of menopause, body mass index (BMI), or lipid profile (total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol). All 66 women had normal weight (BMI range: 18.5–25) and cholesterol levels. The results of OSDI, fluorescein TBUT, corneal fluorescein staining, and Schirmer test differed significantly between the dry eye group and the control group ($P < 0.001$).

3.2. Serum Levels of Sex Steroids. The serum levels of sex steroids in the dry eye group and the control group are

Table 2: Comparison of serum levels of sex steroids between the dry eye group and the normal control group.

Laboratory test	Dry eye group ($n = 44$)	Control group ($n = 22$)	P value
17β-Estradiol (pg/mL)			0.03*
<5	29	20	
≥5	15	2	
Testosterone (nmol/L)	0.60 ± 0.41	0.40 ± 0.24	0.08
DHEA-S (μmol/L)	3.53 ± 1.93	3.15 ± 1.30	0.57
Progesterone (nmol/L)	0.79 ± 0.53	0.63 ± 0.39	0.34

*Pearson's chi-squared test.

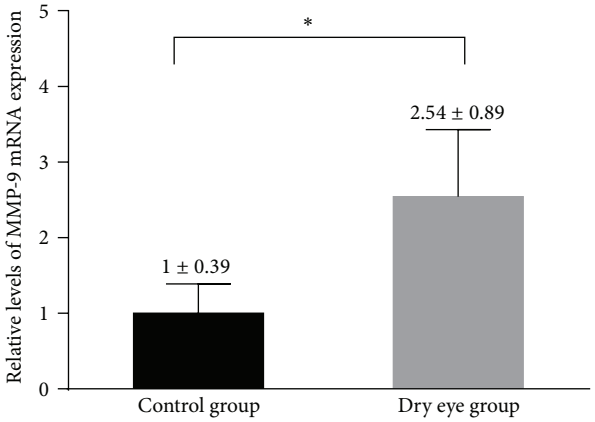

Figure 1: Real-time PCR results of relative levels of MMP-9 mRNA expression in conjunctival cytology samples obtained from patients with dry eye and normal controls. Values were presented as mean ± SD. *Significant difference with $P < 0.05$.

presented in Table 2. This study showed detectable levels of 17β-estradiol (≥5 pg/mL) in 15 dry eye subjects (34% of subjects) and 2 normal controls (9% of subjects). 17β-Estradiol levels were significantly higher in the dry eye subjects than in the controls ($\chi^2 = 4.79$, $P = 0.03$). Levels of testosterone, DHEA-S, and progesterone were higher in the dry eye group, but differences did not reach a level of significance ($P = 0.08$, $P = 0.57$, and $P = 0.34$, resp.).

3.3. MMP-2 and MMP-9 Gene Expression. The results of MMP mRNA expression in conjunctival epithelia were obtained by impression cytology from dry eye subjects and normal controls. Significantly higher levels of MMP-9 mRNA were observed in dry eye subjects than in normal controls ($P = 0.02$, Figure 1). MMP-2 transcripts were undetectable in the samples obtained from normal subjects and those obtained from dry eye subjects.

3.4. MMP-2 and MMP-9 Concentrations in Tears. The concentrations of MMP-2 and MMP-9 in the tears of all subjects are shown in Figure 2. The two MMPs tested were detected in all of the samples. The tear concentrations of MMP-2 and MMP-9 in the dry eye group were significantly greater than those in the control group ($P < 0.001$).

3.5. Correlation of Sex Steroid Levels with MMP-2 and MMP-9 Tear Concentrations and Clinical Tests. Table 3 shows the correlation of sex steroids levels with MMP-2 and MMP-9 tear concentrations and clinical tests in the dry eye group. Specifically, the results of the correlation between 17β-estradiol and other test parameters were calculated from the data gathered from 15 dry eye subjects who had detectable levels of 17β-estradiol. The analysis of the other three sex steroids was conducted in all 44 dry eye subjects. The levels of 17β-estradiol positively correlated with tear concentrations of MMP-2 and MMP-9. The results of Schirmer test showed a significant negative correlation with 17β-estradiol (Figure 3). The levels of testosterone showed a weak negative correlation with TBUT results but no correlation with the results of other tests. The levels of DHEA-S and progesterone showed no correlations with the results of the other tests.

4. Discussion

The prevalence of dry eye is higher in females, especially in postmenopausal women [1]. This fact indicates that sex steroid imbalance is related to the onset and development of dry eye. However, the role of sex steroids in dry eye is complex and remains to be fully understood. Our results demonstrate that the serum levels of 17β-estradiol were significantly higher in postmenopausal women with dry eye than in controls, whereas the levels of testosterone, DHEA-S, and progesterone between the two groups were not significantly different.

Only a few studies have investigated the levels of sex steroids in postmenopausal women with dry eye. Tamer et al. [29] evaluated androgen levels in dry eye patients both with meibomian gland dysfunction (MGD) and without MGD and compared these levels with those of normal control subjects. Total testosterone levels were not significantly different among the three groups, which is consistent with our results. The study also reported lower levels of bioavailable testosterone, DHEA, and DHEA-S in MGD patients than in controls, whereas there was no significant difference between non-MGD dry eye patients and controls. Another small-sample study [30] also found no significant difference in total testosterone levels between postmenopausal women with dry eye and controls. Inconsistent with our findings, a recent study [31] reported that the serum levels of 17β-estradiol and total testosterone were significantly lower in evaporative dry eye patients than in controls. In patients with SS, the disease is not associated with significant alterations in serum levels of testosterone, estrone, or estradiol, whereas DHEA and DHEA-S levels were significantly reduced [32]. However, another study showed no significant difference in DHEA and DHEA-S levels in patients with SS [32, 33].

In our study, we did not subdivide dry eye patients into MGD or non-MGD categories. This could partially explain the differences in sex steroids levels between the current study and previous studies. It would be worth looking at aqueous deficient and evaporative dry eye separately. Other possible factors for the conflicting results among these studies are limited number of subjects, racial differences, and variations in the duration of menopause. In addition, serum sex steroids could not reflect the total estrogen and androgen pool in

Table 3: Spearman correlation of sex steroid levels with tear MMP concentrations and clinical tests.

Parameters	MMP-2		MMP-9		OSDI		TBUT		Corneal staining		Schirmer test	
	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P
17β-Estradiol	0.67	0.006*	0.58	0.03*	−0.07	0.80	−0.38	0.17	−0.09	0.74	−0.58	0.02*
Testosterone	0.28	0.12	0.21	0.24	0.14	0.41	−0.32	0.04*	0.17	0.28	0.02	0.91
DHEA-S	0.004	0.98	0.16	0.38	0.31	0.06	−0.09	0.58	−0.16	0.32	−0.05	0.75
Progesterone	0.07	0.72	0.06	0.77	0.24	0.16	−0.15	0.34	−0.10	0.56	0.02	0.90

DHEA-S = dehydroepiandrosterone sulfate, MMP = matrix metalloproteinase, OSDI = Ocular Surface Disease Index, and TBUT = tear film break-up time.
*Significant difference with $P < 0.05$
ρ: Spearman's correlation coefficient.

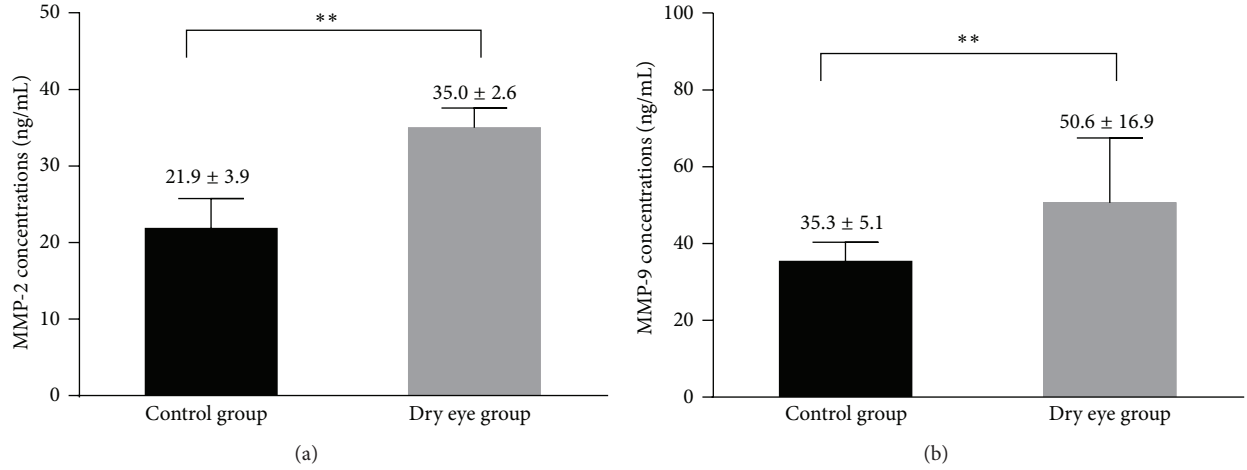

Figure 2: The concentrations of MMP-2 (a) and MMP-9 (b) in tears from patients with dry eye and normal controls. Values were presented as mean ± SD. **Significant difference with $P < 0.001$.

postmenopausal women [34]. It should be noted that humans are unique in possessing adrenal glands that secrete large amounts of DHEA and DHEA-S, which are then converted into androgens and estrogens by steroidogenic enzymes in peripheral tissues and thereby permit target tissues to adjust the amount of active sex hormones according to local requirements [34]. The human ocular surface has been shown to contain mRNAs for steroidogenic enzymes, which are necessary for the local synthesis and metabolism of androgens and estrogens [11]. Therefore, the human ocular surface may be among the many peripheral tissues and be a source of sex steroids.

Versura et al. [6] reported that ocular surface function impairment is greatest when estrogen levels are highest as this impairment occurs during the follicular phase in the menstrual cycle. We found that the levels of estrogen in dry eye patients were still higher than those of age-matched controls after menopause. In another study, 11 out of 20 asymptomatic postmenopausal women developed dry eye symptoms after three months of HRT (estrogen/progesterone) use, whereas symptomatic women were not relieved of dry eye by HRT [35]. A large population-based study of 25,665 postmenopausal women found an increased risk of dry eye in women using HRT, particularly among those using estrogen alone [8]. These data support the hypothesis that estrogen has detrimental effects on the ocular surface.

Inflammation is a common factor that underlies many causes of dry eye. This study assessed the ocular surface expression of MMP-2 and MMP-9, molecules strictly related to the inflammatory process [21]. MMP-9 activity has also been considered to be a better biomarker of dry eye disease severity than traditional clinical signs and is associated with disruption of corneal epithelial barrier function [36, 37].

In this study, quantitative real-time PCR showed that the conjunctival expression of MMP-9 was significantly higher in dry eye patients than in controls, similar to the results of Chotikavanich et al. [36], which found an increasing trend in MMP-9 expression in dry eye subjects stratified by severity level. However, we were unable to detect MMP-2 expression in the conjunctival epithelium in our present study, which is possibly explained by the lower MMP-2 production in the conjunctiva than in other ocular surface tissues [38]. In addition, a lower amount of total RNA was obtained by impression cytology, which may have decreased sensitivity.

Increased MMP-2 and MMP-9 production has been observed in the tear fluid collected from patients with systemic dry eye and from those with nonsystemic dry eye [37, 39]. Consistent with previous findings, we also found that the tear concentrations of MMP-2 and MMP-9 in dry eye subjects were significantly higher than those in controls.

We also observed that the levels of 17β-estradiol were correlated positively with tear concentrations of MMP-2 and

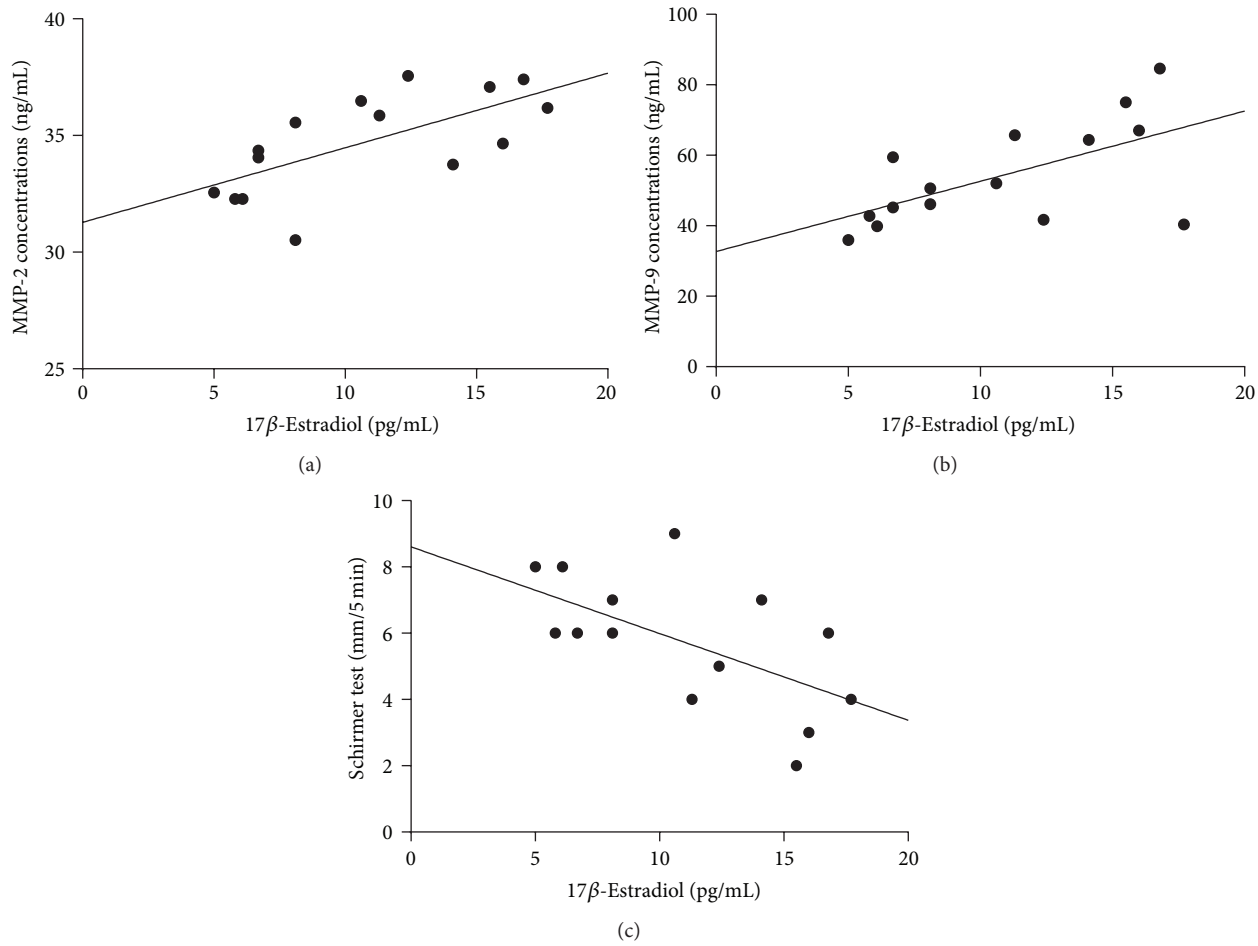

Figure 3: Correlation of 17β-estradiol levels with (a) tear MMP-2 concentrations ($\rho = 0.67$, $P = 0.006$); (b) tear MMP-9 concentrations ($\rho = 0.58$, $P = 0.03$); and (c) Schirmer test results ($\rho = -0.58$, $P = 0.02$). Spearman's rank correlation coefficients were calculated in 15 dry eye subjects who had detectable levels of 17β-estradiol.

MMP-9. This finding indicates that 17β-estradiol stimulates the activity of MMP-2 and MMP-9 in tears of patients with dry eye. The source of tear-derived MMP-2 and MMP-9 has not been established. Corneal epithelial cells [19] and lacrimal glands [21] have been found to synthesize both MMP-2 and MMP-9 in the ocular surface system. A recent study has shown that the major sources of tear-derived MMP-2 and MMP-9 following corneal wounding are the lacrimal gland and conjunctival-associated lymphoid tissue, whereas the corneal epithelium, stromal keratocytes, and conjunctival epithelium including goblet cells contribute little to tear-derived MMP-2 and MMP-9, and the meibomian glands do not appear to contribute at all [38]. Therefore, we postulate that 17β-estradiol may have effects on the promotion of inflammation in the lacrimal gland and conjunctival epithelium and may increase the activity of MMP-2 and MMP-9 in tears of patients with dry eye.

The rationale for our postulation is supported by previous studies on 17β-estradiol effects in ocular surface tissues. Suzuki and Sullivan have reported that 17β-estradiol upregulated the gene expression of proinflammatory cytokines and MMP-2, MMP-7, and MMP-9 in SV40 immortalized human corneal epithelial cells (HCEs) after 6 and/or 24 hours of hormone treatment [20]. However, 17β-estradiol effects on gene expression are not translated into changes in MMP-2 and MMP-9 activity in the culture of either SV40 HCEs or primary corneal epithelial cell cultures [40]. On the other hand, an animal study showed that estrogen treatment of ovariectomized rabbits significantly upregulates the expression and activity of MMP-2 and MMP-9 in the lacrimal gland [21]. A more recent study also demonstrated that systemic estradiol administration increases MMP-2 expression in lacrimal glands of ovariectomized rats [22]. Moreover, in the present study, 17β-estradiol was found to have a negative correlation with Schirmer test results but no correlation with the results of OSDI or corneal staining. Consistent with our result, Mathers et al. also reported a negative correlation between serum estradiol levels and tear production in postmenopausal women [4]. These results indicate that 17β-estradiol leads to regressive, inflammatory changes of the lacrimal gland, and, thus, tear production is reduced.

A limitation of our study is the fact that the method used to assess serum 17β-estradiol levels was not sensitive enough

because many values were below the limit of detection. Further studies with larger subject numbers and with more sensitive analytical techniques are needed.

5. Conclusions

In conclusion, this study demonstrated that serum levels of 17β-estradiol were higher in postmenopausal women with dry eye than in controls. Levels of 17β-estradiol positively correlated with tear MMP-2 and MMP-9 concentrations and negatively correlated with Schirmer test results. We postulate that 17β-estradiol upregulates MMP-2 and MMP-9 production in the lacrimal gland and MMP-9 production in the conjunctival epithelium and thus increases the activity of MMP-2 and MMP-9 in tears of dry eye subjects. Our results support the findings from animal studies that showed upregulation of MMP levels following estradiol treatment.

Disclosure

Guanglin Shen is the first author.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] J. A. Smith, J. Albenz, C. Begley et al., "The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007)," *Ocular Surface*, vol. 5, no. 2, pp. 93–107, 2007.

[2] C. A. McCarty, A. K. Bansal, P. M. Livingston, Y. L. Stanislavsky, and H. R. Taylor, "The epidemiology of dry eye in Melbourne, Australia," *Ophthalmology*, vol. 105, no. 6, pp. 1114–1119, 1998.

[3] E.-M. Chia, P. Mitchell, E. Rochtchina, A. J. Lee, R. Maroun, and J. J. Wang, "Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye study," *Clinical and Experimental Ophthalmology*, vol. 31, no. 3, pp. 229–232, 2003.

[4] W. D. Mathers, D. Stovall, J. A. Lane, M. B. Zimmerman, and S. Johnson, "Menopause and tear function: the influence of prolactin and sex hormones on human tear production," *Cornea*, vol. 17, no. 4, pp. 353–358, 1998.

[5] P. Versura and E. C. Campos, "Menopause and dry eye. A possible relationship," *Gynecological Endocrinology*, vol. 20, no. 5, pp. 289–298, 2005.

[6] P. Versura, M. Fresina, and E. C. Campos, "Ocular surface changes over the menstrual cycle in women with and without dry eye," *Gynecological Endocrinology*, vol. 23, no. 7, pp. 385–390, 2007.

[7] P. Kramer, V. Lubkin, W. Potter, M. Jacobs, G. Labay, and P. Silverman, "Cyclic changes in conjunctival smears from menstruating females," *Ophthalmology*, vol. 97, no. 3, pp. 303–307, 1990.

[8] D. A. Schaumberg, J. E. Buring, D. A. Sullivan, and M. Reza Dana, "Hormone replacement therapy and dry eye syndrome," *The Journal of the American Medical Association*, vol. 286, no. 17, pp. 2114–2119, 2001.

[9] E. M. Rocha, L. A. Wickham, L. A. Da Silveira et al., "Identification of androgen receptor protein and 5α-reductase mRNA in human ocular tissues," *British Journal of Ophthalmology*, vol. 84, no. 1, pp. 76–84, 2000.

[10] B. Esmaeli, J. T. Harvey, and B. Hewlett, "Immunohistochemical evidence for estrogen receptors in meibomian glands," *Ophthalmology*, vol. 107, no. 1, pp. 180–184, 2000.

[11] F. Schirra, T. Suzuki, D. P. Dickinson, D. J. Townsend, I. K. Gipson, and D. A. Sullivan, "Identification of steroidogenic enzyme mRNAs in the human lacrimal gland, meibomian gland, cornea, and conjunctiva," *Cornea*, vol. 25, no. 4, pp. 438–442, 2006.

[12] L. A. Wickham, J. Gao, I. Toda, E. M. Rocha, M. Ono, and D. A. Sullivan, "Identification of androgen, estrogen and progesterone receptor mRNAs in the eye," *Acta Ophthalmologica Scandinavica*, vol. 78, no. 2, pp. 146–153, 2000.

[13] D. A. Sullivan, B. D. Sullivan, M. D. Ullman et al., "Androgen influence on the meibomian gland," *Investigative Ophthalmology & Visual Science*, vol. 41, no. 12, pp. 3732–3742, 2000.

[14] D. A. Sullivan, L. Block, and J. D. O. Pena, "Influence of androgens and pituitary hormones on the structural profile and secretory activity of the lacrimal gland," *Acta Ophthalmologica Scandinavica*, vol. 74, no. 5, pp. 421–435, 1996.

[15] S. Truong, N. Cole, F. Stapleton, and B. Golebiowski, "Sex hormones and the dry eye," *Clinical and Experimental Optometry*, vol. 97, no. 4, pp. 324–336, 2014.

[16] P. Versura, G. Giannaccare, and E. C. Campos, "Sex-steroid imbalance in females and dry eye," *Current Eye Research*, vol. 40, no. 2, pp. 162–175, 2015.

[17] K.-S. Na, D. H. Jee, K. Han, Y.-G. Park, M. S. Kim, and E. C. Kim, "The ocular benefits of estrogen replacement therapy: a population-based study in postmenopausal Korean women," *PLoS ONE*, vol. 9, no. 9, Article ID e106473, 2014.

[18] A. A. Jensen, E. J. Higginbotham, G. M. Guzinski, I. L. Davis, and N. J. Ellish, "A survey of ocular complaints in postmenopausal women," *Journal of the Association for Academic Minority Physicians*, vol. 11, no. 2-3, pp. 44–49, 2000.

[19] D.-Q. Li, B. L. Lokeshwar, A. Solomon, D. Monroy, Z. Ji, and S. C. Pflugfelder, "Regulation of MMP-9 production by human corneal epithelial cells," *Experimental Eye Research*, vol. 73, no. 4, pp. 449–459, 2001.

[20] T. Suzuki and D. A. Sullivan, "Estrogen stimulation of proinflammatory cytokine and matrix metalloproteinase gene expression in human corneal epithelial cells," *Cornea*, vol. 24, no. 8, pp. 1004–1009, 2005.

[21] C. Zylberberg, V. Seamon, O. Ponomareva, K. Vellala, M. Deighan, and A. M. Azzarolo, "Estrogen up-regulation of metalloproteinase-2 and -9 expression in rabbit lacrimal glands," *Experimental Eye Research*, vol. 84, no. 5, pp. 960–972, 2007.

[22] X. Song, P. Zhao, G. Wang, and X. Zhao, "The effects of estrogen and androgen on tear secretion and matrix metalloproteinase-2 expression in lacrimal glands of ovariectomized rats," *Investigative Ophthalmology and Visual Science*, vol. 55, no. 2, pp. 745–751, 2014.

[23] C. S. Wingrove, E. Garr, I. F. Godsland, and J. C. Stevenson, "17β-Oestradiol enhances release of matrix metalloproteinase-2 from human vascular smooth muscle cells," *Biochimica et*

Biophysica Acta (BBA)—Molecular Basis of Disease, vol. 1406, no. 2, pp. 169–174, 1998.

[24] M. Potier, S. J. Elliot, I. Tack et al., "Expression and regulation of estrogen receptors in mesangial cells: influence on matrix metalloproteinase-9," *Journal of the American Society of Nephrology*, vol. 12, no. 2, pp. 241–251, 2001.

[25] R. M. Schiffman, M. D. Christianson, G. Jacobsen, J. D. Hirsch, and B. L. Reis, "Reliability and validity of the ocular surface disease index," *Archives of Ophthalmology*, vol. 118, no. 5, pp. 615–621, 2000.

[26] M. A. Lemp, "Report of the National Eye Institute/Industry workshop on clinical trials in dry eyes," *The CLAO Journal*, vol. 21, no. 4, pp. 221–232, 1995.

[27] W. L. Miller and R. J. Auchus, "The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders," *Endocrine Reviews*, vol. 32, no. 1, pp. 81–151, 2011.

[28] R. Singh, A. Joseph, T. Umapathy, N. L. Tint, and H. S. Dua, "Impression cytology of the ocular surface," *British Journal of Ophthalmology*, vol. 89, no. 12, pp. 1655–1659, 2005.

[29] C. Tamer, H. Oksuz, and S. Sogut, "Androgen status of the nonautoimmune dry eye subtypes," *Ophthalmic Research*, vol. 38, no. 5, pp. 280–286, 2006.

[30] M. C. B. Duarte, N. T. Pinto, H. Moreira, A. T. R. Moreira, and D. Wasilewski, "Total testosterone level in postmenopausal women with dry eye," *Arquivos Brasileiros De Oftalmologia*, vol. 70, no. 3, pp. 465–469, 2007.

[31] C. Gagliano, S. Caruso, G. Napolitano et al., "Low levels of 17-β-oestradiol, oestrone and testosterone correlate with severe evaporative dysfunctional tear syndrome in postmenopausal women: a case-control study," *British Journal of Ophthalmology*, vol. 98, no. 3, pp. 371–376, 2014.

[32] D. A. Sullivan, A. Bélanger, J. M. Cermak et al., "Are women with Sjögren's syndrome androgen-deficient?" *Journal of Rheumatology*, vol. 30, no. 11, pp. 2413–2419, 2003.

[33] M. T. Brennan, V. Sankar, R. A. Leakan et al., "Sex steroid hormones in primary Sjögren's syndrome," *The Journal of Rheumatology*, vol. 30, no. 6, pp. 1267–1271, 2003.

[34] F. Labrie, A. Bélanger, V. Luu-The et al., "DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging," *Steroids*, vol. 63, no. 5-6, pp. 322–328, 1998.

[35] U. Erdem, O. Ozdegirmenci, E. Sobaci, G. Sobaci, U. Göktolga, and S. Dagli, "Dry eye in post-menopausal women using hormone replacement therapy," *Maturitas*, vol. 56, no. 3, pp. 257–262, 2007.

[36] S. Chotikavanich, C. S. de Paiva, de Quan Li et al., "Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome," *Investigative Ophthalmology & Visual Science*, vol. 50, no. 7, pp. 3203–3209, 2009.

[37] K. R. Vandermeid, S. P. Su, K. W. Ward, and J.-Z. Zhang, "Correlation of tear inflammatory cytokines and matrix metalloproteinases with four dry eye diagnostic tests," *Investigative Ophthalmology and Visual Science*, vol. 53, no. 3, pp. 1512–1518, 2012.

[38] A. Petznick, M. C. Madigan, Q. Garrett, D. F. Sweeney, and M. D. M. Evans, "Contributions of ocular surface components to matrix-metalloproteinases (MMP)-2 and MMP-9 in feline tears following corneal epithelial wounding," *PLoS ONE*, vol. 8, no. 8, Article ID e71948, 2013.

[39] A. Solomon, D. Dursun, Z. Liu, Y. Xie, A. Macri, and S. C. Pflugfelder, "Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease," *Investigative Ophthalmology and Visual Science*, vol. 42, no. 10, pp. 2283–2292, 2001.

[40] T. Suzuki and D. A. Sullivan, "Comparative effects of estrogen on matrix metalloproteinases and cytokines in immortalized and primary human corneal epithelial cell cultures," *Cornea*, vol. 25, no. 4, pp. 454–459, 2006.

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Research Article

Evaluation of Dry Eye and Meibomian Gland Dysfunction in Teenagers with Myopia through Noninvasive Keratograph

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Purpose. This study aims to evaluate dry eye and ocular surface conditions of myopic teenagers by using questionnaire and clinical examinations. *Methods*. A total of 496 eyes from 248 myopic teenagers (7–18 years old) were studied. We administered Ocular Surface Disease Index (OSDI) questionnaire, slit-lamp examination, and Keratograph 5M. The patients were divided into 2 groups based on OSDI dry eye standard, and their ocular surfaces and meibomian gland conditions were evaluated. *Results*. The tear meniscus heights of the dry eye and normal groups were in normal range. Corneal fluorescein scores were significantly higher whereas noninvasive break-up time was dramatically shorter in the dry eye group than in the normal group. All three meibomian gland dysfunction parameters (i.e., meibomian gland orifice scores, meibomian gland secretion scores, and meibomian gland dropout scores) of the dry eye group were significantly higher than those of the normal group ($P < 0.0001$). *Conclusions*. The prevalence of dry eye in myopic teenagers is 18.95%. Meibomian gland dysfunction plays an important role in dry eye in myopic teenagers. The Keratograph 5M appears to provide an effective noninvasive method for assessing ocular surface situation of myopic teenagers.

1. Introduction

Dry eye disease is defined by the Report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop as a multifactorial disease of tears and ocular surface, which results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface [1]. Dry eye is a common ocular surface disease that often occurs in the elderly [2]. More than 20% of people in 30–40-year-olds have dry eye, and the prevalence of dry eye in people over 70 years old is as high as 36.1% [3]. Currently, with the increasing popularity of computers, video games, and smartphones in the younger generation, the incidence of myopia in teenagers is increasing annually, with a growing number of myopic teenagers exhibiting frequent blinking, sensitivity to light, and other dry eye ocular discomfort [4]. Dry eye is of an increasingly important clinical significance in myopic adolescents as it affects their quality of life. Diagnosis of dry eye currently relies on break-up time (BUT) and Schirmer's tests. However, BUT speed is different for different people. Moreover, fluorescein sodium affects the tear film's stability. BUT and Schirmer's tests are both invasive examinations. Adolescents are more difficult to evaluate than adults for ocular surface dysfunction because of poorer compliance with the procedure. Thus the traditional diagnostic methods for identifying dry eye in adolescents are less definitive since children are more sensitive to the procedure than adults. Accordingly, the data reproducibility is more variable making it more difficult to identify the disease signs in an adolescent population. Accordingly, reported dry eye incidence in myopics is underdiagnosed. Given the lower prevalence of dry eye disease in children, the diagnosis of dry eye is often overlooked by many ophthalmologists [5]. Previous studies have confirmed that Keratograph 5M (Oculus, Wetzlar, Germany) noninvasively measures noninvasive break-up time (NIBUT), tear meniscus height, and meibography with low irritability [6–10]. Therefore, in this study, we used Keratograph 5M combined

with slit-lamp examination and dry eye questionnaire to give myopic adolescents a series of dry eye-related inspections and assessments and to determine the prevalence of dry eye and ocular surface conditions among myopic adolescents.

2. Materials and Methods

2.1. Materials. A total of 248 consecutive patients (average age 12.26 ± 1.86 years, range 7–18 years; 132 female, 116 male, male to female ratio = 1 : 1.14) who went to Tianjin Medical University Eye Hospital myopia clinic from January to June in 2014 with no systemic or ocular treatment, contact lens wear, keratitis, ocular allergic disease, any other ocular surface disease, glaucoma, active and chronic uveitis, or previous ocular surgery or injury were recruited in this prospective study.

Written informed consent was obtained from the parents of the patients. The study was approved by the Institutional Review Board of the Tianjin Medical University Eye Hospital and performed in accordance with the tenets of the Declaration of Helsinki.

2.2. Methods. This study was a prospective study, and all inspections were performed by the same experienced examiner.

2.2.1. Questionnaire Regarding Dry Eye. Before clinical examination, each patient completed an Ocular Surface Disease Index (OSDI) questionnaire for assessment of ocular surface symptoms and the severity of dry eye. This questionnaire [11] included questions regarding the frequency of dry eye symptoms experienced in the previous week (light sensitivity, gritty sensation, painful or sore eyes, blurred vision, and poor vision), vision-related daily activities (reading, watching TV, working on computers, and driving at night), and environmental triggers (wind, air conditioning, and low humidity). Each answer was scored on a 5-point scale (all of the time: 4, most of the time: 3, half of the time: 2, some of the time: 1, and none of the time: 0), and the OSDI score was calculated as follows: {(sum of scores × 25)/total number of questions}. Thus, the total OSDI score ranged from 0 to 100. A higher OSDI score represented greater disability. Answering was completed with the assistance of one doctor, and the completion time was controlled within 4–6 min. Currently, no uniform national standards have been established for the diagnosis of dry eye, and the diagnostic criteria are inconsistent worldwide. Based on their OSDI scores, the patients were categorized as having a normal ocular surface (0–12 points) or as having mild (13–22 points), moderate (23–32 points), or severe (33–100 points) ocular surface disease [12]. The study population was divided into normal and dry eye groups, which included those with mild dry eye, moderate dry eye, and severe dry eye. The two groups were compared to assess their ocular surface conditions.

2.2.2. Keratograph 5M: Noninvasive Measurement for Ocular Surface. Keratograph 5M inspection items include noninvasive tear film break-up time, noninvasive tear meniscus height, and meibography. The tests were first measured in the right eye and then the left eye. Three measurements were taken, and the average of results was considered in the statistics.

Keratograph 5M was used to grade the right eyelid using the following meibomian gland dropout degrees as meiboscore [13]: Grade 0: no loss of meibomian gland; Grade 1: loss of < 1/3 of the whole gland area; Grade 2: loss of 1/3-2/3 of the whole gland area; and Grade 3: loss of > 2/3 of the whole gland area. The meiboscore of each eye was calculated as the sum of the scores from both upper and lower eyelids, making the total meiboscore per eye in a range of 0–6.

2.2.3. Slit-Lamp Examination of the Anterior Segment. The following examinations were carried out sequentially using a slit-lamp: meibomian gland orifices, meibomian gland lipid secretion, and corneal fluorescein staining scores.

The quality of the meibomian gland orifices was scored semiquantitatively in the central eight glands of the lower right eyelid as follows: Grade 0 is normal, that is, no obstruction of orifice and being covered with a thin and smooth fluid; Grade 1 is obstruction of one or two meibomian gland orifices or secretions or occlusion; Grade 2 is obstruction of two or three meibomian gland orifices with thick fluid; Grade 3 is obstruction or narrowing of almost half of the meibomian gland orifices; Grade 4 is obstruction or narrowing of more than half of the meibomian gland orifices with sticky secretions.

The quality of the meibum was scored semiquantitatively in the central eight glands of the lower right eyelid as follows (0–24 points in total) [14]: Grade 0: clear fluid; Grade 1: cloudy fluid; Grade 2: cloudy, particulate fluid; and Grade 3: inspissated, toothpaste-like fluid.

Corneal fluorescein staining was graded from 0 to 12, which was a sum of the scores of corneal four quadrants scored individually as 0 (no staining), 1 (mild staining with a few scattered dots of stains), 2 (moderate staining between 1 and 3), and 3 (severe staining with confluent stains or corneal filaments) [15].

2.3. Statistical Analysis. Statistical analysis was performed using SPSS version 19.0. All variables were expressed as the mean ± standard deviation. Indexes were analyzed using nonparametric Mann-Whitney U test, and the intergroup data were compared using Shapiro-Wilk test. Spearman correlation analysis was used to estimate the correlations between various factors. Categorical variables were compared between the groups using the chi-square test. The confidence interval was set at 95%, and probability values of $P < 0.05$ were considered statically significant.

3. Results

3.1. Dry Eye Detection Rate. A total of 248 subjects (496 eyes, average age 12.26 ± 1.86 years) were recruited for the study. A total of 116 males (average age 11.9 ± 2.55 years) and 132 females (average age 12.2 ± 2.45 years) participated.

OSDI screened out 201 normal people (81.05%), 23 mild dry eye people (9.27%), 15 moderate dry eye people (6.05%),

Table 1: Comparison of general condition and ocular surface parameters between the dry eye group and the normal group.

Group	Dry eye	Normal	P
Age (year)	12.45 ± 1.54	11.75 ± 1.95	0.051
Sex ratio (male/female)	25/22	98/103	0.175
OSDI	27.02 ± 14.35	7.29 ± 3.36	<0.001
Tear meniscus height (mm)	0.23 ± 0.03	0.22 ± 0.03	0.214
NIBUT (s)	6.32 ± 2.49	13.14 ± 3.67	<0.001
Corneal fluorescein scores	3.51 ± 1.67	1.23 ± 2.32	<0.0001

Table 2: Comparison of meibomian gland functional indexes between the dry eye group and the normal group.

Group	Dry eye	Normal	P
Meibomian gland orifice scores	1.82 ± 0.53	0.51 ± 0.62	<0.0001
Meibomian gland secretion scores	1.35 ± 0.59	0.41 ± 0.35	<0.0001
Meibomian gland dropout scores	3.21 ± 1.02	0.61 ± 0.65	<0.0001

and 9 severe dry eye people (3.63%). Based on the OSDI dry eye standard, 47 (18.95%) dry eye populations were detected. The right eyes of the 47 dry eye patients were included in the dry eye group (25 males and 22 females) and the right eyes of 201 normal eye patients were included in the normal group (98 males and 103 females). Statistical comparison of the two groups was then carried out.

3.2. Comparison of General Condition and Ocular Statistical Indexes between the Dry Eye Group and the Normal Group. Table 1 shows that no significant differences in age, gender, and tear meniscus height were found between the dry eye and the normal groups. Tear meniscus height was normal for both groups (>0.20 mm), with 0.23 ± 0.03 mm in the dry eye group and 0.22 ± 0.03 mm in the normal group.

The average score of OSDI of the dry eye group was 27.02 ± 14.35, and the average score of corneal fluorescein in the dry eye group was 3.51 ± 1.67. The average score of corneal fluorescein in the normal group was 7.29 ± 3.36 and the average score of corneal fluorescein in the normal group was 1.23 ± 2.32. These two indicators were significantly higher in the dry eye group than in the normal group ($P < 0.001$). The average of NIBUT in the dry eye group was 6.32 ± 2.49 and was significantly lower than that of the normal group, which was 13.14 ± 3.67 ($P < 0.001$).

3.3. Comparison of Meibomian Gland Indexes between the Dry Eye Group and the Normal Group. In contrast with the normal group, the meibomian gland orifice scores, meibomian gland secretion scores, and meibomian gland dropout scores were significantly higher in the dry eye group ($P < 0.0001$) (Table 2).

3.4. Correlation Analyses between Scores of Complaining of Dry Eye and Ocular Surface Analysis Indicators. A highly significant inverse correlation was observed between the value of OSDI and NIBUT (rs = −0.982, $P = 0.000$) (Figure 1). Moreover, a highly significant correlation was observed between the value of OSDI and meibomian gland dropout scores (rs = 0.838, $P = 0.000$) (Figure 2).

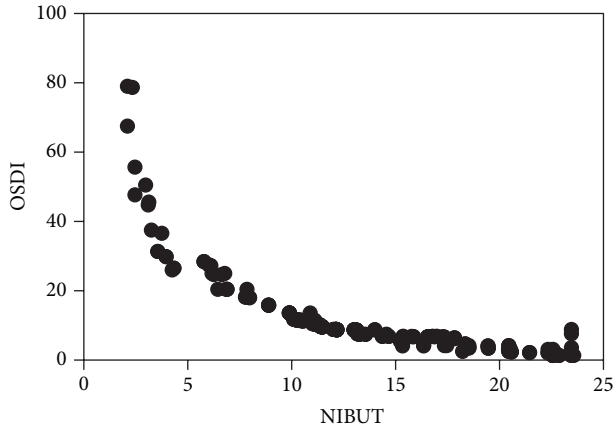

Figure 1: Correlation analysis between NIBUT and OSDI. Negative correlation was found between NIBUT and OSDI in the two groups.

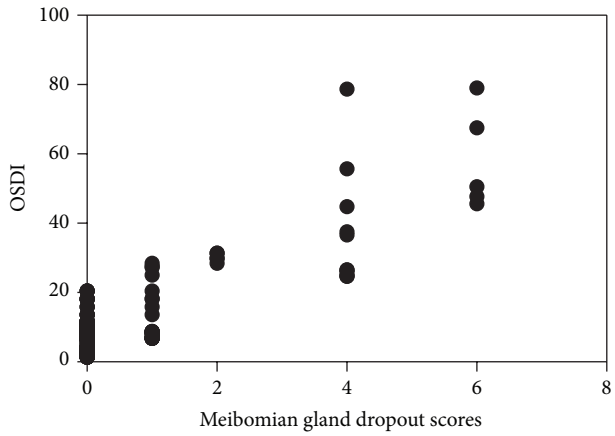

Figure 2: Correlation analysis between meibomian gland dropout scores and OSDI. Positive correlation was found between meibomian gland dropout scores and OSDI in the two groups.

4. Discussion

Recent studies showed that dry eye is a major clinical problem affecting quality of life [4] as it reduces the immunity of ocular surface, causes eye symptoms in children, leads to visual fluctuations during the day, and affects visual clarity in the daytime. Moreover, dry eye can reduce learning efficiency in children. Dry eye is widely believed to be a type of disease whose incidence increases with age [5], and thus scholars have conducted much dry eye research for the elderly. The ability of children to express eye symptoms are worse than adults, or some children may be able to express it clearly but dry eye examinations are difficult. Moreover, allergic conjunctivitis has a higher prevalence in children, and many

children who have this condition also suffer from dry eye, making dry eye diagnosis more difficult [16]. Thus, the dry eye incidence in children was underestimated by many scholars. In this study, we use Keratograph 5M combined with slit-lamp examination and dry eye questionnaire to give myopic adolescents a series of dry eye-related inspections and assessments. Dry eye incidence in children was found to be 18.95% which is lower than that in adults but still not significant. Undiagnosed dry eye can lead to fragile ocular surface environment, irreversible eye damage, and increased possibility of corneal ulcers and scars [5]. Accurate diagnosis, systemic treatment, and etiological control can improve eye health and ensure good visual quality in young people.

Keratograph 5M is an objective, comprehensive, and noninvasive dry eye diagnostic device that can detect NIBUT, noninvasive tear meniscus height, and meibomian gland dropout. Keratograph 5M exhibits high accuracy in the dry eye diagnosis in adults [17]. The current study shows that Keratograph 5M has a good implementation even in children, and it can be combined with questionnaire to facilitate clinical diagnosis of dry eye in children. OSDI, NIBUT, and meibomian gland dropout are correlated to dry eye in adolescents, which means that aggravated dry eye symptoms are associated with worse unstable tear film and increased meibomian gland dropout. The lower prevalence of dry eye disease in children relative to adults, limitations of diagnosis, lower degree of the subjective assessment of symptoms in children, and the lack of clinician attention reduce dry eye awareness.

The meibomian glands are the main source of lipids for human tear film. The lipid layer of the tear film slows evaporation of the aqueous of tear film, preserves a clear optical surface, and forms a barrier to protect the eye from microbial agents and organic matter [18]. The meibomian gland plays a more important role than aqueous tear volume in determining the severity of ocular discomfort and dry eye conditions [19]. Lipid-deficient dry eye caused by meibomian gland dysfunction (MGD) has increasingly drawn ophthalmologists' attention. MGD is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction or qualitative/quantitative changes in the glandular secretions. MGD may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease [20]. MGD could reduce tear film stability and cause ocular complaints, inflammation, and other ocular surface disorders [21]. The mean values of tear meniscus height in the dry eye and the normal groups were both in the normal range, whereas NIBUT in the dry eye group was shorter than that of the normal group, which suggests that the dry eye group has normal tear volume but relatively unstable tear film relative to the normal group. The dry eye group of myopic teenagers has a high corneal staining score, more abnormality of meibomian gland orifices and meibomian gland lipid secretions, and more meibomian gland dropouts, causing serious MGD. This result is similar to that of previous studies where lack of meibomian gland is also accompanied by damaged meibomian gland function [7]. This result implies that the common type of dry eye among myopic teenagers is lipid abnormalities of dry eye (i.e., evaporative dry eye). Currently, the clinical evaluation of dry eye is mainly based on BUT and Schirmer tests, whereas the evaluation of meibomian gland function and lipid layer is deficiency. Keratograph 5M, which has a high compatibility in children, has been found to provide early diagnostic and therapeutic values in children for the diagnosis of meibomian gland function and tear film stability. Combined with the questionnaire, the ratio of failure diagnosis of dry eye in children can be reduced.

Currently, the main correction methods of juvenile myopia are frame glasses, contact lens, and orthokeratology (ortho-k). The effectiveness of overnight orthokeratology in flattening the cornea and temporarily reducing myopia has been widely documented [22]. Parents increasingly choose night-wear ortho-k to control myopia of their children. Given that ortho-k is placed on the cornea for the whole night, the ocular surface condition of adolescents with refractive errors should be fully assessed. When considering adolescent ortho-k treatment, we should also pay attention to the situation of the ocular surface of the patients, especially meibomian gland function and dry eye prevalence, which can help improve the safety of the treatment.

The clinical and epidemiological aspects of dry eye in children have not been as well described as in adults [5]. The prevalence of dry eye disease in children varies greatly depending on which criteria and methods were used in previous research. Reportedly, 9.7% of all children have been diagnosed with dry eye disease [4]. Dry eye disease associated with longtime reading can have many signs and symptoms involved, a lot of which are still not understood. Many Chinese children with arduous learning tasks have experienced these signs and symptoms. Myopia has been associated with strenuous near task as well. Blink rates during near work are decreased leading to improper tear film placement. In this study, only normal myopic adolescents were chosen to analyze dry eye and ocular surface. The results suggest that the prevalence of dry eye in adolescents with myopia is 18.95% higher than other research documents entail. For further study regarding dry eye disease in children expanding the number of patients and the inclusion of emmetropes adolescents should be considered.

Conflict of Interests

None of the authors has conflict of interests related to the paper.

Authors' Contribution

Xiu Wang and Xiaoxiao Lu contributed to the work equally and should be regarded as co-first authors.

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References

[1] "The definition and classification of dry eye disease: report of the definition and classification subcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[2] S. E. Moss, R. Klein, and B. E. K. Klein, "Long-term incidence of dry eye in an older population," *Optometry and Vision Science*, vol. 85, no. 8, pp. 668–674, 2008.

[3] A. Sahai and P. Malik, "Dry eye: prevalence and attributable risk factors in a hospital-based population," *Indian Journal of Ophthalmology*, vol. 53, no. 2, pp. 87–91, 2005.

[4] J. H. Moon, M. Y. Lee, and N. J. Moon, "Association between video display terminal use and dry eye disease in school children," *Journal of Pediatric Ophthalmology and Strabismus*, vol. 51, no. 2, pp. 87–92, 2014.

[5] M. Alves, A. C. Dias, and E. M. Rocha, "Dry eye in childhood: epidemiological and clinical aspects," *Ocular Surface*, vol. 6, no. 1, pp. 44–51, 2008.

[6] Y. Jiang, H. Ye, J. Xu, and Y. Lu, "Noninvasive Keratograph assessment of tear film break-up time and location in patients with age-related cataracts and dry eye syndrome," *Journal of International Medical Research*, vol. 42, no. 2, pp. 494–502, 2014.

[7] D. Finis, P. Ackermann, N. Pischel et al., "Evaluation of meibomian gland dysfunction and local distribution of meibomian gland atrophy by non-contact infrared meibography," *Current Eye Research*, vol. 40, no. 10, pp. 982–989, 2015.

[8] S. Koh, C. Ikeda, S. Watanabe et al., "Effect of non-invasive tear stability assessment on tear meniscus height," *Acta Ophthalmologica*, vol. 93, no. 2, pp. e135–e139, 2015.

[9] W. Ngo, S. Srinivasan, M. Schulze, and L. Jones, "Repeatability of grading meibomian gland dropout using two infrared systems," *Optometry and Vision Science*, vol. 91, no. 6, pp. 658–667, 2014.

[10] N. Best, L. Drury, and J. S. Wolffsohn, "Clinical evaluation of the Oculus Keratograph," *Contact Lens and Anterior Eye*, vol. 35, no. 4, pp. 171–174, 2012.

[11] F. Özcura, S. Aydin, and M. R. Helvaci, "Ocular surface disease index for the diagnosis of dry eye syndrome," *Ocular Immunology and Inflammation*, vol. 15, no. 5, pp. 389–393, 2007.

[12] K. L. Miller, J. G. Walt, D. R. Mink et al., "Minimal clinically important difference for the ocular surface disease index," *Archives of Ophthalmology*, vol. 128, no. 1, pp. 94–101, 2010.

[13] R. Arita, K. Itoh, S. Maeda et al., "Proposed diagnostic criteria for obstructive meibomian gland dysfunction," *Ophthalmology*, vol. 116, no. 11, pp. 2058–2063.e1, 2009.

[14] A. Tomlinson, A. J. Bron, D. R. Korb et al., "The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 4, pp. 2006–2049, 2011.

[15] A. A. Afonso, D. Monroy, M. E. Stern, W. J. Feuer, S. C. G. Tseng, and S. C. Pflugfelder, "Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms," *Ophthalmology*, vol. 106, no. 4, pp. 803–810, 1999.

[16] M. M. Hom, A. L. Nguyen, and L. Bielory, "Allergic conjunctivitis and dry eye syndrome," *Annals of Allergy, Asthma and Immunology*, vol. 108, no. 3, pp. 163–166, 2012.

[17] S. Srinivasan, K. Menzies, L. Sorbara, and L. Jones, "Infrared imaging of meibomian gland structure using a novel keratograph," *Optometry and Vision Science*, vol. 89, no. 5, pp. 788–794, 2012.

[18] K. B. Green-Church, I. Butovich, M. Willcox et al., "The international workshop on meibomian gland dysfunction: report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease," *Investigative Ophthalmology and Visual Science*, vol. 52, no. 4, pp. 1979–1993, 2011.

[19] H. Wu, Y. Wang, N. Dong et al., "Meibomian gland dysfunction determines the severity of the dry eye conditions in visual display terminal workers," *PLoS ONE*, vol. 9, no. 8, Article ID e105575, 2014.

[20] K. K. Nichols, G. N. Foulks, A. J. Bron et al., "The international workshop on meibomian gland dysfunction: executive summary," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 4, pp. 1922–1929, 2011.

[21] G. Geerling, J. Tauber, C. Baudouin et al., "The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction," *Investigative Ophthalmology and Visual Science*, vol. 52, no. 4, pp. 2050–2064, 2011.

[22] C. Maldonado-Codina, S. Efron, P. Morgan, T. Hough, and N. Efron, "Empirical versus trial set fitting systems for accelerated orthokeratology," *Eye & Contact Lens*, vol. 31, no. 4, pp. 137–147, 2005.

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Research Article

The Effects of Hemodialysis on Tear Osmolarity

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Aim. To determine the effects of hemodialysis (HD) on tear osmolarity and to define the blood biochemical tests correlating with tear osmolarity among patients with end stage renal disease (ESRD). *Material-Method*. Tear osmolarity of ESRD patients before and after the hemodialysis program was determined as well as the blood biochemical data including glucose, sodium, potassium, calcium, urea, and creatinine levels. *Results*. Totally 43 eyes of 43 patients (20 females and 23 males) with a mean age of 53.98 ± 18.06 years were included in the study. Tear osmolarity of patients was statistically significantly decreased after hemodialysis (314.06 ± 17.77 versus 301.88 ± 15.22 mOsm/L, $p = 0.0001$). In correlation analysis, pre-HD tear osmolarity was negatively correlated with pre-HD blood creatinine level ($r = -0.366$, $p = 0.016$). Post-HD tear osmolarity was statistically significantly correlated with the post-HD glucose levels ($r = 0.305$ $p = 0.047$). Tear osmolarity alteration by HD was negatively correlated with creatinine alteration, body weight alteration, and ultrafiltration ($r = -0.426$, $p = 0.004$; $r = -0.365$, $p = 0.016$; and $r = -0.320$, $p = 0.036$, resp.). There was no correlation between tear osmolarity and Kt/V and URR values. *Conclusion*. HD effectively decreases tear osmolarity to normal values and corrects the volume and composition of the ocular fluid transiently. Tear osmolarity alteration induced by HD is correlated with body weight changes, creatinine alterations, and ultrafiltration.

1. Introduction

Hemodialysis (HD) is the main treatment method in patients with end stage renal disease (ESRD) to correct the composition and volume of body fluids. The adequacy of HD is still a main subject for active investigation [1, 2].

There are some parameters present for evaluation of adequacy of HD such as Kt/V (a number used to quantify hemodialysis treatment adequacy, in which K is the dialyzer clearance of urea, t is the dialysis time, and V is the volume of distribution of urea) and urea reduction ratio (URR) [3].

HD may alter the volume and composition of ocular fluid as well as the systemic hemodynamic parameters [4]. Presence of dry eye in patients with ESRD is known for years [5–7].

Dry eye disease is an important and common public health problem with 5–35% prevalence in general population, as it causes discomfort and deterioration in quality of vision [8]. Although, a direct method is still not present, dry eye questionnaires, Schirmer's test, and tear break-up time (TBUT) are in clinical use to support the dry eye diagnosis. Nowadays, tear osmolarity measurement is regarded as the most accurate way of diagnosis of dry eye disease [9]. As previous studies showed, reduction in aqueous tear flow as a result of lacrimal failure with or without accelerated evaporation from the tear film is major determinants of the tear hyperosmolarity [8, 10]. Additionally, the electrolytes of the aqueous phase of the tear film can effect tear osmolarity [11]. Of the electrolytes present in the tear film, cations sodium and potassium (120–170 mmol/kg and 6–42 mmol/kg, resp.) and anions chloride and bicarbonate (106–135 mmol/kg and 26 mmol/kg, resp.) are the major contributors to tear osmolarity [12]. Measurement of tear osmolarity in clinical setting

is easy with recently developed lab-on-a-chip technology, namely, TearLab (TearLab Corporation, San Diego, CA, USA), with 72.8% sensitivity and 92.0% specificity at a cutoff value of 312 mOsms/L [9].

In this study, we aimed to determine the effects of HD, performed with isovolemic and standard sodium (Na^+) (138 mEq/L) and potassium (K^+) (2 Eq/L) containing dialysates, on tear osmolarity and to evaluate the correlation between blood biochemical tests and tear osmolarity in patients with ESRD.

2. Material and Method

2.1. Patients. Tear osmolarity of 43 eyes of 43 patients under the regular, 3 times per week, hemodialysis program in Bagcilar Education and Research Hospital, Hemodialysis Unit, was evaluated and McMonnies and Ho questionnaire was filled in between April 2014 and June 2014. The blood samples were taken and tear osmolarity was detected one minute before the beginning of the hemodialysis and 30 minutes after the termination of hemodialysis program. Patients with diabetic retinopathy and any rheumatic and connective tissue diseases, patients using any type of eye drops and wearing contact lenses, and patients with the history of ocular surgery were excluded from the study.

The study protocol was approved by the local ethics committee in Bagcilar Education and Training Hospital, Turkey. Informed consent was obtained from all subjects.

2.2. Laboratory Tests. Pre-HD blood samples were taken and tear osmolarity was detected one minute before the beginning of HD. The rate of diffusion and blood flow between body compartments reduce the effective K and therefore Kt/V and result in the postdialysis rebound. To take account of these factors, Kt/V should ideally be calculated using a postdialysis sample taken 30–60 minutes after dialysis when the urea concentrations have reequilibrated [13]. As urea enters into tear fluid by simple diffusion [14], post-HD tear osmolarity was detected and biochemical samples were taken 30 minutes after the termination of HD session. Body weights of participants were recorded as well as the ultrafiltration amount. Serum urea, creatinine, glucose, sodium, potassium, calcium, and bicarbonate (HCO_3^-) levels were studied using the standard methods recommended by the manufacturer. Serum osmolarity was calculated with the formula [15]:

$$\text{Serum osmolarity} = 2\left[Na^+\right] + \frac{[\text{Glucose}]}{18} + \frac{[\text{Blood urea nitrogen}]}{2.8}. \quad (1)$$

The urea reduction ratio (URR) is a number used to quantify dialysis treatment adequacy and similarly Kt/V is also a number used to quantify hemodialysis treatment adequacy, in which K is the dialyzer clearance of urea, t is the dialysis time, and V is the volume of distribution of urea.

Table 1: Clinical characteristics of the patients.

Gender (F/M)	20/23
Age (years)	53.98 ± 18,06
Time of dialysis (years)	5.12 ± 5.67
Presence of hypertension	32 (76%)
Presence of diabetes mellitus	16 (38%)
Mean URR (%)	73.52 ± 6.1
Mean spKt/V	1.42 ± 0.16

URR was calculated as a percentage of post-HD blood urea nitrogen (BUN) divided by pre-HD BUN. The single-pool Kt/V delivered by hemodialysis was estimated by the second-generation Daugirdas equation [16].

We have recorded the URR and Kt/V and investigated their associations with tear osmolarity.

Dry eye symptoms of the patients are evaluated with McMonnies and Ho questionnaire. Any score over 14.5 indicates a strong likelihood of dry eye disease [17]. Tear osmolarity was measured using lab-on-a-chip technology TearLab Osmolarity System (TearLab Corporation, 9980 Huennekens Street, Ste 100, San Diego, CA 92121, 1-855-832-7522, USA), one minute before the beginning of HD and 30 minutes after the termination of HD. The measurements were performed at a stable room temperature of 25–25.5°C and the room humidity was 50–55%. Quality control procedures were applied at the beginning of each day of patient testing by using reusable electronic check cards (provided by the manufacturer as a procedural quality control) to confirm the function and calibration of the TearLab Osmolarity System. A tear sample, approximately 50 nL, was collected from the inferior lateral tear meniscus of the ocular surface by the same investigator.

2.3. Statistical Analysis. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 21.0. Descriptive statistics were summarized as mean ± SD or percentage. Paired samples t-test was performed in comparison of pre-HD and post-HD results. Chi square test was used in comparison of two groups. Pearson's correlation analysis was performed to determine the correlations of laboratory data with tear osmolarity. A p value of <0.05 was regarded as statistically significant.

3. Results

Totally, 43 eyes of 43 patients (20 females and 23 males) with a mean age of 53.98 ± 18.06 years were included in the study.

McMonnies and Ho questionnaire was performed before HD to 41 patients because 2 patients were unable to complete the questionnaire. Mean questionnaire score was 6.27 ± 5.02. And all patients gave the same answers to the questionnaire after HD. There was only one patient who had dry eye according to the McMonnies and Ho questionnaire.

Descriptive characteristics of study participants are summarized in Table 1.

In the 37 of the 43 patients (86%) URR was over 65% and Kt/V was over 1.2 and HD was adequate according to these two parameters.

Table 2: The laboratory data analysis of patients before and after HD.

	Before HD	After HD	p
Body weight	70.59 ± 15.95	68.54 ± 15.57	**0.0001**
Tear osmolarity (mOsm/L)	314.05 ± 17.77	301.88 ± 15.22	**0.0001**
Serum glucose (mg/dL)	128.63 ± 92.26	121.77 ± 42.66	0.533
Serum urea (mg/dL)	130.96 ± 31.03	34.88 ± 12.4	**0.0001**
Serum creatinine (mg/dL)	8.08 ± 2.36	2.87 ± 1.01	**0.0001**
Serum calcium (mEq/L)	8.79 ± 1,16	9.43 ± 0.71	**0.0001**
Serum sodium (mEq/L)	137.28 ± 3.73	136.47 ± 2.69	0.165
Serum potassium (mEq/L)	5.22 ± 0.79	3.96 ± 0.66	**0.0001**
Serum HCO_3^- (mEq/L)	20.59 ± 1.8	23.1 ± 3.5	**0.0001**

The data are reported in mean ± standard deviation. Results of paired samples t-test.

Body weight, serum urea, creatinine, calcium, and potassium, and tear osmolarity of patients statistically decreased, and HCO_3^- increased significantly after HD ($p = 0.0001$) (Table 2).

There was no significant correlation between pre-HD tear osmolarity and pre-HD serum osmolarity, glucose, urea, sodium, potassium, calcium, and bicarbonate levels and body weight ($p > 0.05$). Pre-HD tear osmolarity was statistically significantly correlated with pre-HD creatinine ($r = -0.366$ $p = 0.016$).

There was no significant correlation between post-HD tear osmolarity and post-HD serum urea, creatinine, sodium, potassium, calcium, and bicarbonate levels and body weight ($p > 0.05$). Post-HD tear osmolarity was statistically significantly correlated with post-HD glucose ($r = 0.305$, $p = 0.047$).

We also subgrouped patients according to the presence of diabetes mellitus type 2 (DM) and hypertension (HT). The difference regarding pre-HD and post-HD tear osmolarity between patients with or without DM was not statistically significant ($p > 0.05$) (Table 3).

The difference regarding pre-HD tear osmolarity between patients with or without HT was not statistically significant ($p > 0.05$). But post-HD tear osmolarity of patients without HT was statistically significantly lower than patients with HT ($p = 0.043$) (Table 4).

In correlation analysis, tear osmolarity difference was statistically significantly correlated with ultrafiltration, body weight difference, and creatinine difference but not with URR and Kt/V values. The p values of correlation analysis are summarized in Table 5.

Table 3: Effects of DM on pre-HD and post-HD tear osmolarity.

	DM	n	Mean ± SD	p
Pre-HD tear osmolarity	DM (−)	11	319.18 ± 18.8	0.655
	DM (+)	7	323.43 ± 21.10	
Post-HD tear osmolarity	DM (−)	11	302.10 ± 14.06	0.413
	DM (+)	7	308.86 ± 20.23	

Table 4: Effects of HT on pre-HD and post-HD tear osmolarity.

	HT	n	Mean ± SD	p
Pre-HD tear osmolarity	HT (−)	10	311.40 ± 16.77	0.482
	HT (+)	27	316.00 ± 17.71	
Post-HD tear osmolarity	HT (−)	10	295.50 ± 11.21	**0.043**
	HT (+)	27	306.29 ± 14.66	

Table 5: Correlation analysis.

		Tear osmolarity difference
Ultrafiltration	r	−0.320
	p	**0.036**
Body weight difference	r	−0.365
	p	**0.016**
Creatinine difference	r	−0.426
	p	**0.004**
URR	r	0.057
	p	0.718
Kt/V	r	0.055
	p	0.728

The results of Pearson's correlation analysis. r: correlation coefficient; p: statistical significance.

4. Discussion

The adequacy of HD is important for management of patients with ESRD. According to latest guidelines the minimally adequate dose of HD given 3 times per week to patients with K_r less than 2 mL/min/1.73 m^2 should be spKt/V of 1.2 per dialysis. For treatment less than 5 hours, an alternative minimum dose is URR of 65% [3]. In our study HD achieved minimally adequate doses in 37 patients (86%) but not in 6 patients.

In an adequate hemodialysis serum sodium levels are determined as 135–145 mEq/L, potassium 3–9 mEq/L, calcium 7–12 mEq/L, bicarbonate > 15 mEq/L, creatinine < 12 mEq/L, and albumin >3 gr/dL [3]. In our study all of these biochemical markers were in these ranges.

In this study, we have evaluated tear osmolarity of patients with ESRD one minute before the beginning of HD and 30 minutes after the end of HD. We observed tear hyperosmolarity before HD and a significant reduction to normal levels after HD (314.05 ± 17.77 mOsm/L and 301.88 ± 15.22 mOsm/L, resp., $p < 0.0001$). Gilbard et al. indicate in two rabbit models for keratoconjunctivitis sicca that decreased tear volume or excessive evaporation is the major cause of tear hyperosmolarity [10]. Charlton et al. report tear hyperosmolarity, using freezing point depression method, (average 347 mOsm/L, range 375–312 mOsm/L) in 10 renal dialysis patients in pre-HD and tested 5 of them immediately after completion of HD. They show a significant reduction in tear osmolarity after HD in all patients, correlating with our results. They speculate that, from the three principle solutes (sodium, glucose, and urea), urea is the only one that freely passes from serum to the tears and responsible for tear hyperosmolarity in renal dialysis patients [18].

In our study only one patient scored positively for dry eye before HD according to the McMonnies and Ho questionnaire and all patients gave the same answers to the questionnaire after HD. Similarly, Charlton et al. report that none of the renal dialysis subjects scored positively for dry eye. They speculate that, from the three principle solutes (sodium, glucose, and urea), urea is the only one that freely passes from serum to the tears and is responsible for tear hyperosmolarity in renal dialysis patients. According to them, hemodialysis patients remain asymptomatic for dry eye mainly because of the protective effects of urea in tears on the ocular surface [18].

In this study, we did not find any correlation between serum electrolyte levels and tear osmolarity both in pre-HD and post-HD periods. Aktaş et al. determined a prognostic importance of serum calcium levels for the ocular findings and symptoms in patients with ESRD [5]; however, we did not determine any correlation between pre-HD and post-HD calcium levels and tear osmolarity. Serum osmolarity also did not correlate with tear osmolarity.

There was no significant correlation between pre-HD tear osmolarity and pre-HD glucose and urea levels and body weight ($p > 0.05$). Pre-HD tear osmolarity was statistically significantly correlated with pre-HD creatinine ($r = -0.366$, $p = 0.016$). This is the first report denoting an association between tear osmolarity and creatinine according to our knowledge.

There was no significant correlation between post-HD tear osmolarity and post-HD serum urea and creatinine levels and body weight ($p > 0.05$). Post-HD tear osmolarity was statistically significantly correlated with post-HD glucose ($r = 0.305$ $p = 0.047$).

In correlation analysis tear osmolarity difference was statistically significantly correlated with ultrafiltration ($r = -0.320$, $p = 0.036$), body weight difference ($r = -0.365$, $p = 0.016$), and creatinine difference ($r = -0.426$, $p = 0.004$).

In another study among patients undergoing HD for ESRD, the incidence of reduced basal tear secretion and dry eye symptoms were reported to be higher in diabetic patients than in nondiabetics [19]. However, in our study, we have subgrouped patients according to the presence of DM and we did not determine any significant effects of diabetes in pre-HD or post-HD tear osmolarity. Post-HD tear osmolarity of patients with HT was statistically significantly higher than patients without HT ($p = 0.043$). Studies with larger sample sizes are needed for exact relevance of these results.

Jung et al. reported decrease in tear break-up time (TBUT) and Schirmer's tests after HD [20]. We did not evaluated Schirmer's test and TBUT because of low patient compliance after HD.

This study has some limitations. As HD is a long lasting treatment method, patients may be divided into subgroups according to duration of HD. We have evaluated patients regardless of the duration of HD. Lack of Schirmer's test and TBUT are two other limitations. We could not do Schirmer's test and TBUT because of low patient compliance after HD. Kt/V was <1.2 and URR was <65% and HD was not adequate in only 6 patients. More patients are needed to assess the association between tear osmolarity and adequacy of HD.

In conclusion tear osmolarity is correlated with the serum creatinine levels in pre-HD period. In post-HD period, tear osmolarity is correlated with serum glucose levels. Tear osmolarity alteration induced by HD is correlated with body weight changes, creatinine alterations, and ultrafiltration. Therefore, HD corrects the volume and composition of the ocular fluid transiently.

Disclosure

None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the results.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] J. E. Flythe, G. C. Curhan, and S. M. Brunelli, "Shorter length dialysis sessions are associated with increased mortality, independent of body weight," *Kidney International*, vol. 83, no. 1, pp. 104–113, 2013.

[2] P. G. Blake, "Adequacy of dialysis revisited," *Kidney International*, vol. 63, no. 4, pp. 1587–1599, 2003.

[3] Hemodialysis Adequacy 2006 Work Group, "Clinical practice guidelines for hemodialysis adequacy, update 2006," *American Journal of Kidney Diseases*, vol. 48, pp. S2–S90, 2006.

[4] R. D. Evans and M. Rosner, "Ocular abnormalities associated with advanced kidney disease and hemodialysis," *Seminars in Dialysis*, vol. 18, no. 3, pp. 252–257, 2005.

[5] S. Aktaş, H. M. Sağdık, H. Aktaş et al., "Tear function in patients with chronic renal failure undergoing hemodialysis," *Renal Failure*, vol. 37, no. 2, pp. 245–248, 2015.

[6] A. Akinci, N. Cakar, N. Kara, and N. Uncu, "Ocular findings in children with chronic renal failure," *Cornea*, vol. 28, no. 1, pp. 5–6, 2009.

[7] M. R. Manaviat, M. Rashidi, M. Afkhami-Ardekani, and M. R. Shoja, "Prevalence of dry eye syndrome and diabetic retinopathy in type 2 diabetic patients," *BMC Ophthalmology*, vol. 8, article 10, 2008.

[8] International Dry Eye WorkShop, "The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007)," *The Ocular Surface*, vol. 5, no. 2, pp. 75–92, 2007.

[9] M. A. Lemp, A. J. Bron, C. Baudouin et al., "Tear osmolarity in the diagnosis and management of dry eye disease," *The American Journal of Ophthalmology*, vol. 151, no. 5, pp. 792–798, 2011.

[10] J. P. Gilbard, S. R. Rossi, K. L. Gray, L. A. Hanninen, and K. R. Kenyon, "Tear film osmolarity and ocular surface disease in two rabbit models for keratoconjunctivitis sicca," *Investigative Ophthalmology and Visual Science*, vol. 29, no. 3, pp. 374–378, 1988.

[11] J. Murube, "Tear osmolarity," *Ocular Surface*, vol. 4, no. 2, pp. 62–73, 2006.

[12] U. Stahl, M. Willcox, and F. Stapleton, "Osmolality and tear film dynamics," *Clinical & Experimental Optometry*, vol. 95, no. 1, pp. 3–11, 2012.

[13] A. T. Azar, "Adaptive network based on fuzzy inference system for equilibrated urea concentration prediction," *Computer Methods and Programs in Biomedicine*, vol. 111, no. 3, pp. 578–591, 2013.

[14] J. H. Thaysen and N. A. Thorn, "Excretion of urea, sodium, potassium and chloride in human tears," *The American Journal of Physiology*, vol. 178, no. 1, pp. 160–164, 1954.

[15] L. I. Worthley, M. Guerin, and R. W. Pain, "For calculating osmolality, the simplest formula is the best," *Anaesthesia & Intensive Care*, vol. 15, no. 2, pp. 199–202, 1987.

[16] J. T. Daugirdas, "Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error," *Journal of the American Society of Nephrology*, vol. 4, no. 5, pp. 1205–1213, 1993.

[17] C. McMonnies, A. Ho, and D. Wakefield, "Optimum dry eye classification using questionnaire responses," in *Lacrimal Gland, Tear Film, and Dry Eye Syndromes 2*, vol. 438 of *Advances in Experimental Medicine and Biology*, pp. 835–838, Springer, 1998.

[18] J. F. Charlton, I. R. Schwab, and R. Stuchell, "Tear hyperosmolarity in renal dialysis patients asymptomatic for dry eye," *Cornea*, vol. 15, no. 4, pp. 335–339, 1996.

[19] M. Nakata, Y. Okada, H. Kobata et al., "Diabetes mellitus suppresses hemodialysis-induced increases in tear fluid secretion," *BMC Research Notes*, vol. 7, article 78, 2014.

[20] J. W. Jung, M. H. Yoon, S. W. Lee, and H. S. Chin, "Effect of hemodialysis (HD) on intraocular pressure, ocular surface, and macular change in patients with chronic renal failure. Effect of hemodialysis on the ophthalmologic findings," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 251, no. 1, pp. 153–162, 2013.