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Letters to the Editor

Author Response: Concerns About a Dog Model of Dry Eye Disease

We appreciate the comments raised in the Letter to the Editor from Dr. Ofri et al.[1] The concerns largely stem from a misunderstanding of the original article[17] and an oversimplified description of the postoperative care regimen.

The authors of the Letter to the Editor first challenged the scientific merit of establishing a surgically induced dry eye model, because keratoconjunctivitis sicca, known as *dry eye disease* (DED), is a common and spontaneous canine disease. They also questioned whether other existing animal models could serve the purpose to evaluate therapeutic modalities for DED. To address these concerns, we would like to provide more background on the use of salivary gland transplantation as a treatment modality for DED and the remaining challenges. Current therapies involving tear replacement products or punctal plugs could not adequately address the clinical challenges of severe DED. Salivary gland transplantation is only indicated in patients with absolute aqueous tear deficiency (Schirmer's test <2 mm) with persistent severe discomfort and when all other means have failed. Its possible indications can include cicatricial conjunctivitis (such as Stevens-Johnson syndrome, chemical burns), surgical damage, or radioablation of lacrimal tissue.[2] We believe that minor salivary gland (MSG) transplantation offers promising therapeutic potential. In fact, our research group has accumulated abundant experience in the autotransplantation of submandibular glands and minor salivary glands in clinical treatment of severe DED.[3–6] Based on our previous results, 60% to 83.3% of patients received promising relief of symptoms after MSG transplantation, but a minority was not satisfied with the long-term efficacy.[6] To this end, an appropriate animal model is necessary to explore the mechanisms of hyposecretion and testing the novel approaches to increase secretion of transplanted MSG. Unfortunately, however, the existing animal models are not suitable for evaluation of MSG transplantation. As stated in the introduction of our original article, the naturally-occurring, spontaneous DED model is more suitable for the therapeutic research of DED related to autoimmune diseases such as Sjögren's syndrome. But since these autoimmune conditions affect salivary gland function and cause xerostomia,[7] this model is not suitable for our purpose. This is further supported by our clinical experience from the past two decades investigating the effect of salivary gland transplantation to treat severe DED.[4–6] In clinical practice, as reported by our group and others, DED patients with autoimmune diseases are often unsuitable candidates for salivary gland transplantation because of gland damage.[6,8] Hence, we deemed the spontaneous keratoconjunctivitis sicca model inappropriate. In addition, other animal species also face various shortcomings based on the anatomical structures of lacrimal glands. In mice and rabbits, the palperal fissures are too small for surgery or observation. In miniature pigs, the texture of the oral mucosa is too hard and caused a foreign object sensation in recipient eyes. On the other hand, Beagle dogs possess large palpebral fissures, soft mucosal graft, and high densities of MSG.[9,10] Therefore, establishing a lacrimal gland ablation canine DED model, rather than using rabbit DED model, spontaneous DED model, or conducting premature clinical trials, is scientifically justified.

The authors of the Letter to the Editor next challenged the novelty of our animal model, suggesting that it is similar to the first lacrimal gland ablation DED model introduced by Helper et al.[11] in 1974. In the Helper model, the orbital lacrimal glands and nictitans lacrimal glands were removed while preserving the third eyelid. However, this DED model presented noticeable individual variations in Schirmer test and ocular surface inflammation.[12] In our study, we sought to establish a more reproducible and robust DED model. We used a mature experimental canine strain with stable ocular anatomy and modified the previous surgical techniques by removing the orbital lacrimal glands and the entire third eyelid. In our model, removal of the third eyelid completely purged the nictitans lacrimal glands and eliminated its defensive functions, which closely simulated the environmental stress in human eyes.[13] Overall, our reported

animal model represents a significant departure from the technique published in 1974.

The authors of the letter demonstrated significant concerns for the animal welfare during our experiments. We believe this stems from a misunderstanding based on the omissions in our description of the postoperative analgesic usage in the original article. To rectify this issue, we have included additional experimental details in an Erratum.[14] Briefly, the animal research protocols were approved by our institutional ethics committee, and the experiments were conducted in the Laboratory Animal Center, where qualified veterinarians performed maintenance and administration of anesthesia, antibiotics, and analgesics. We have provided documented evidence of verification by the IACUC and Laboratory Animal Center staff that the experimental procedures including the postoperative care regimen was strictly enforced according to the approved protocol, in adherence to ARRIVE guidelines and the ARVO Statement for Use of Animals. Documented log of analgesics and antibiotics used was also provided to the editors for review. To alleviate surgical pain and reduce ocular discomfort in animals, immediately after the surgical procedures, antibiotics (penicillin, 40,000 units/kg, intramuscularly) and analgesics (carprofen, 4 mg/kg, orally) were administered daily for a period of three days. Additionally, eye drops with 3% levofloxacin were given three times daily for five days after operation. Ocular surface care such as cleanup of excess discharge was performed once daily for the first month after surgery and once weekly thereafter. To avoid additional discomfort while animals were healing from surgery, we waited three weeks before performing tests for tear breakup time and corneal fluorescein staining. For Schirmer test, because the testing required less intervention or animal restraint, we performed the test earlier (one week after operation), with all animals cooperating. All tests were performed as gently as possible to avoid causing discomfort or fear in canine subjects. None of canine subject was purposely placed in pain because of their lack of cooperation.

Additionally, we would like to clarify that, after the initial three days of postoperative pain/infection control, we did not use analgesics for continued pain management. This was based on human clinical guidelines for treating dry eye patients. According to the consensus report from TFOS DEWS II, neither topical nor systemic analgesics are included in the current treatment and management options for DED.[2] We also consulted experienced ophthalmologists regarding the use of analgesics for treating dry eye patients, and none of the physicians stated that analgesics were the first-line medication for DED. The nature of the pain (nociceptive or neuropathic) primarily caused by DED and its underlying mechanism remain unclear,[15] and an analgesic that could effectively alleviate dry eye–related pain is still under development.[16] Considering the lack of support by evidence-based research and the objectivity of our observational experiments, no analgesics were used during the observation period.

At the end of the observation period, aside from one canine subject that was euthanized to harvest specimens, MSG transplantation was immediately performed on all other subjects to alleviate dry eye symptoms. Schirmer test, tear film breakup time, and the inflammation assessment indicated that the dry eye symptoms should have significantly improved after transplantation.

We appreciate the concerns and discussions of Dr. Ofri et al.[1] and could not agree more that animal welfare is of utmost importance. We have provided compelling clarifications on the scientific rigor for our animal model and assurance that we are equally uncompromising in protecting the welfare of laboratory animals in our present and future work.

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References

1. Ofri R, Millichamp NJ, Keller C, et al. Concerns about a dog model of dry eye disease. *Transl Vis Sci Technol*. 2024;13(3):28, doi:10.1167/tvst.13.3.28.
2. Jones L, Downie LE, Korb D, et al. TFOS DEWS II management and therapy report. *Ocul Surf*. 2017;15:575–628.
3. Su JZ, Zheng B, Liu XJ, et al. Quality of life and patient satisfaction after submandibular gland transplantation in patients with severe dry eye disease. *Ocul Surf*. 2019;17:470–475.
4. Zhang L, Su JZ, Cai ZG, et al. Factors influencing the long-term results of autologous microvascu-

lar submandibular gland transplantation for severe dry eye syndrome. *Int J Oral Maxillofac Surg*. 2019;48:40–47.
5. Su JZ, Wang Z, Liu XJ, et al. Use of saliva flow rate measurement in minor salivary glands autotransplantation for treatment of severe dry eye disease. *Br J Ophthalmol*. 2022;106:902–907.
6. Su JZ, Zheng B, Wang Z, et al. Submandibular gland transplantation vs minor salivary glands transplantation for treatment of dry eye: a retrospective cohort study. *Am J Ophthalmol*. 2022;241:238–247.
7. Quimby FW, Schwartz RS, Poskitt T, et al. A disorder of dogs resembling Sjögren's syndrome. *Clin Immunol Immunopathol*. 1979;12:471–476.
8. Wang Z, Li W, Hong X, et al. Minor salivary glands function is decreased in hyposalivation-related diseases. *Arch Oral Biol*. 2016;69:63–70.
9. Gabner S, Michels C, Lanz B, et al. Labial and buccal minor salivary glands of the dog - location, three-dimensional arrangement and histology. *Vet Ophthalmol*. 2021;24:400–407.
10. Castanho LS, Moreira H, Ribas C, et al. Labial salivary glands transplantation in the treatment of dry eye in dogs by autograft. *Rev Bras Oftalmol*. 2013;72:373–378.
11. Helper LC, Magrane WG, Koehm J, et al. Surgical induction of keratoconjunctivitis sicca in the dog. *J Am Vet Med Assoc*. 1974;165:172–174.
12. Helper LC. The effect of lacrimal gland removal on the conjunctiva and cornea of the dog. *J Am Vet Med Assoc*. 1970;157:72–75.
13. Saito A, Watanabe Y, Kotani T. Morphologic changes of the anterior corneal epithelium caused by third eyelid removal in dogs. *Vet Ophthalmol*. 2004;7:113–119.
14. Li Z, Zou Y, Zhu H, et al. Erratum in: Establishment of a Beagle dog model of dry eye disease. *Transl Vis Sci Technol*. 2024;13(3):27, doi:10.1167/tvst.13.3.27.
15. Belmonte C, Nichols JJ, Cox SM, et al. TFOS DEWS II pain and sensation report. *Ocul Surf*. 2017;15:404–437.
16. Tei Y, Mikami Y, Ito M, et al. Pathogenic mechanism of dry eye-induced chronic ocular pain and a mechanism-based therapeutic approach. *Invest Ophthalmol Vis Sci*. 2022;63:7.
17. Li Z, Zou Y, Zhu H, et al. Establishment of a Beagle dog model of dry eye disease. *Transl Vis Sci Technol.* 2023;12(1):2, doi:10.1167/tvst.12.1.2.