Animal models of dry eye disease: Useful, varied and evolving (Review)

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Received April 7, 2021; Accepted June 10, 2021

DOI: 10.3892/etm.2021.10830

Abstract. Dry eye disease (DED), which is a prevalent disease that still lacks successful treatment options, remains a major challenge in ophthalmology. Multiple animal models of DED have been used to decipher its pathophysiology and to develop novel treatments. These models use mice, rats, rabbits, cats, dogs and non-human primates. Each model assesses aspects of DED by focusing on elements of the lacrimal functional unit, which controls the homeostasis of the tear film. The present review outlines representative DED animal models and assesses their contribution to the study of DED. Murine models are the most extensively used, followed by rabbit models; the latter offer the advantage of larger eyes, a favorable biochemical profile for drug studies, experimental ease and relatively low cost, contrasting with non-human primates, which, although closer to humans, are not as accessible and are expensive. No comprehensive 'ideal' animal model encompassing all aspects of human DED exists nor is it feasible. Investigators often choose an animal model based on their experimental needs and the following four features of a given model: The size of the eye, its biochemical composition, the available research reagents and cost. As research efforts in DED expand, more refined animal models are needed to supplement the enormous contribution made to date by existing models.

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Key words: dry eye disease, Sjogren's syndrome, lacrimal glands, meibomian glands, animal models

1. Introduction

Animal models of diseases often help investigators to decipher their pathogenesis or develop new treatments. Dry eye disease (DED), a multifactorial disease of the ocular surface that for years lacked even a precise definition, remains a major research challenge in ophthalmology (1). Its importance derives mainly from its high prevalence, variously reported to affect between 5 and 15% of the world population, and from the lack of treatments that provide, at a minimum, adequate symptomatic control.

Homeostasis of the ocular surface maintains key components of ocular function, notably an optically clear cornea and the normal composition of the tear film, which protects the ocular surface from inflammation, infection, desiccation, and traumatic injury. The lacrimal functional unit, thought to maintain such homeostasis, consists of the conjunctival and corneal epithelia, the lacrimal and Meibomian glands, the interconnecting innervation, and the hormonally responsive and immune cells resident in these tissues. Assessing the anatomical and functional elements of the lacrimal functional unit has contributed to our understanding of DED.

Recent progress in the pathophysiology of DED, classifying it as aqueous-deficient, evaporative or mixed, provides a useful reference point to categorize and assess the animal models that have been developed over the years (Table I). It is perhaps superfluous to state at the outset that no animal model can ever recapitulate the entirety of a human disease. Nevertheless, investigators developing such models aim to capture components of the target disease that determine its pathophysiology and response to novel treatments.

DED models have employed a wide range of species, extending from mice to nonhuman primates. It is only a matter of time before zebra fish are used in DED studies, given its potential contribution to understanding the function of the anterior chamber (2). The contributions

of existing models, along with their limitations, provide the impetus for their further refinement until the medical frontier of DED is finally conquered. Here, we present the main murine, rat, rabbit, cat, dog and non-human primate models of this disease and discuss persistent needs in model development.

2. Mouse models of DED

Mouse models. Mouse models of DED are the most commonly used mainly because mice are small, easy to handle, and inexpensive to house. Additional reasons for their widespread use are the availability of interesting knockout and transgenic strains, and of immunological and molecular reagents for their study. Below, we describe the main types of mouse DED models.

Mouse models of Sjögren syndrome. Sjögren syndrome, the most common autoimmune disease in humans, is characterized by mononuclear cell infiltration of the salivary and lacrimal glands (LGs) (3,4). There are several mouse models of Sjögren syndrome, each recapitulating one or more of its aspects, but none is perfect.

The *MRL/lpr mouse*, primarily used to study systemic lupus erythematosus, was found to have coexisting Sjögren syndrome (5). Similar to humans, inflammation is significantly greater in lacrimal and salivary glands of female MRLP/lpr mice compared to age-matched males (6). The onset of dacryoadenitis is observed at the age of 1 month, and the disease progresses rapidly, with the mice dying at 6 months (7). The MRL/lpr mice spontaneously develop T-cell-derived LG inflammation, mimicking the human disorder. The T cells, most of them CD4 T cells, represent approximately 80% of the inflammatory cells in LG lesions (8,9). The mutation of the lpr gene results alters the Fas protein, causing lymphoproliferation with defective lymphocyte apoptosis, clonal deletion of autoreactive T cells in peripheral lymphoid organs, and elimination of activated T cells.

The *non-obese diabetic (NOD) mouse*, first established as a model of insulin-dependent diabetes mellitus, shows secondary autoimmune dacryoadenitis, in which predominantly CD4+Th1 cells infiltrate the LGs (10-12). A variant strain of the NOD mouse (NOD.B10.H2^{b} with an altered major histocompatibility complex region) does not develop diabetes. However, because of LG T cell infiltration, it displays a mild Sjögren phenotype at the age of 10 weeks, followed by severe dacryoadenitis and DED at 1 year (13). Interestingly, in male mice dacryoadenitis has a higher incidence and develops earlier compared to females. These differences have been attributed to differences in sex steroid hormones (14).

There are several additional Sjögren syndrome models, such as the *NZB/NZW F1* (15,16), the *NFS/sld* (17), the *neurturin-deficient* (18), the *TGF-β1 knockout* (19-21), the *IQI/jic* (21), and the *Id3-deficient* (22) mice. Each mouse model exhibits a unique characteristic of dacryoadenitis making them suitable for the study of particular aspects of Sjögren syndrome related DED. Strain-specific characteristics of the disease enable targeted manipulation of mechanisms underlying the autoimmune process.

Lacrimal gland excision model. Shinomiya's group developed an exorbital LG excision mouse model (an aqueous-deficient DED model) with persistent tear volume reduction, superficial punctate keratitis, and increased tear levels of IL-1β. Unfortunately, the inflammatory infiltrate of the ocular surface tissues was not sufficiently pronounced to make this model informative. Thus, the added intraorbital LG excision, which led to severe tear volume reduction and severe inflammatory changes in the corneal surface. They also devised a minimally invasive approach to remove the intraorbital LGs via the subcutaneous tissue of the temporal lid margin, which greatly increased the success rate of the surgery (23,24).

Desiccating environmental stress model. Ocular surface desiccation is considered one of the initiating factors in DED (25). Pflugfelder's group was the first to develop a model in which ocular surface desiccation was an initiating factor of DED (26), an evaporative DED model. The environmental desiccating stress is generated by exposing the eyes of mice to a constant low-humidity air flow aimed at the face for 4 h every day. The longer the exposure to desiccating stress, the more prolonged the decreased tear secretion and the longer the recovery time. For example, if mice were exposed to the stress for 4 h per day for 1, 3 or 10 days, decreased tear production lasted for 2, 6 and 18 days, respectively, after discontinuing the desiccating stress (27). When the environmental stress is combined with muscarinic blockade to further reduce tear secretion the resulting DED is even more pronounced (7,26,28-31). Muscarinic blockade is often achieved by subcutaneous administration of scopolamine, which acts on the LGs to reduce tear secretion (7,25). The desiccation plus scopolamine more faithfully represents features of aqueous-deficient DED, including reductions in tear production, tear film stability, corneal staining, conjunctiva goblet cells, and increases in apoptosis of ocular surface epithelium and tear cytokines levels (32-35).

Modifications of this model have been introduced to generate chronic DED, akin to the clinical condition in humans. DED is induced by applying desiccating stress as above for 14 days and transferring the mice to an environment with normal humidity for 4 months (no muscarinic blockade is needed). The severity of DED peaks on day 14. The chronic phase is characterized by corneal epitheliopathy and inflammation, which persists for a long time, never returning to normal (36).

It is worth mentioning the different mechanisms by which desiccation and muscarinic blockade induce DED. Desiccation induces greater conjunctival CD3 (+) T-cell infiltration, and higher Th17-cell activity and Treg dysfunction than muscarinic blockade, while muscarinic blocked decreases tear volume more than desiccation, attenuates Th17 activity and enhances Th2 and Treg responses without affecting Th1 activity. There is increasing support for the combination of desiccation with muscarinic blockade than for either agent alone (25,37).

Models based on the aging of mice. Since age is a significant risk factor for DED (38,39), investigators have produced DED models based on their aging. C57BL/6 mice have been used successfully to study age-related chronic DED, which

develops spontaneously in mice over 1 year old, which is characterized by a combination of DED and Meibomian gland dysfunction (MGD). This is thought to reflect the condition in humans older than 50 years with DED and MGD. Highlights of this model are increased production of MMP-9 and T-cell related cytokines in the ocular surface, and influx of $CD4^+$ and $CD8^+$ T cells into aged LG (40-42). There is also Meibomian gland dropout, increased meibocyte differentiation, and increased expression of cytokines. This model is suitable to the study the pathogenesis of age-related MGD (43-45). Pathophysiologically, they represent mixed models.

3. Rat models of DED

Rats, being bigger than mice, have relatively larger eyes, while at the same time these animals are still easy to handle and relatively inexpensive to maintain. Such difference in size, although not great, is nevertheless enough to comfortably perform functional assessments and morphological and molecular analyses of the eye. Not surprisingly, in many cases mouse models have inspired the corresponding rat models and vice versa.

Scopolamine model. A popular rat model has been based on the scopolamine mouse model presented above, an aqueous-deficient DED model. Rats were given subcutaneous scopolamine and housed in an environmentally controlled room with the standard temperature and low humidity (25±2%) for 28 days (46-48). The experiment rats displayed reduced tear production, reduced tear film stability, increased corneal fluorescent staining, and decreased conjunctiva goblet cells (48,49).

Visual display terminal user model. The computer and visual display terminal syndrome is a constellation of ocular (and extraocular) symptoms associated with prolonged use of visual display terminals (50,51). Evaporative DED is part of this syndrome, which is gaining importance with the widespread use of such technologies. Decreased blink frequency, the result of intense attention to the display screen, is considered central to its pathogenesis.

Investigators have established a rat dry eye model of corneal epithelial disorders by inducing improper tear dynamics and changes in blink frequency (52-54). To simulate the video display terminal, the rats are housed under continuous exposure to low-humidity airflow and placed on a balance swing for 7.5 h per day. These rats showed chronic reduction of tear secretion, impaired LG function with abnormal morphology, and superficial punctate keratopathy similar to that in humans. Potentially protective agents have already been tried, a topic of growing importance in a society dependent on computers for daily life functions (53).

Additional models. Several other rat DED models, some analogous to mouse models, have been reported but experience with them is limited or their technical description lacks detail. They include models generated by: Dacryoadenectomy (55-57); orchiectomy or ovariectomy (58,59); autoimmune dacryoadenitis induced by injecting LG extract (60,61); injection of botulinum B into the LG (62); exposure to tobacco smoke (63); and exposure to urban particulate matter (64).

4. Rabbit models of DED

Rabbits have significant advantages over rats and mice for the study of DED. Their larger globe size entails a larger exposed ocular surface, which makes it much easier to perform an array of DED tests such as the Schirmer tear test, tear break up time, fluorescein, rose Bengal staining, and corneal sensitivity using esthesiometry. In addition, as mentioned, rabbits are suitable to the study of drug molecules susceptible to hydrolysis by ocular surface esterase (65).

At least 12 novel rabbit models of DED have been reported. The majority of them attempt to reduce tear production by either removing the LGs or impeding their function. The most direct approaches include partial surgical resection of the LG (with or without concurrent removal of the nictitating membrane and Harderian gland) (66) or closure of the LG excretory ducts with cautery (67). Impairing LG function has been done by: Irradiation of the LGs (68); induction of dacryoadenitis by injecting the LGs with activated lymphocytes (69) or the plant mitogen Concanavalin A into one (70) or all (71) orbital LGs; injection of botulinum toxin A to the palpebral portion of the superior LG (72); or LG denervation (73). Pharmacological agents such as topical atropine (74) or benzalkonium chloride (75) have also been used to induce primarily aqueous-deficient DED in rabbits. Other methods include closing the Meibomian gland openings by cauterization (76); acute desiccation of the eye by manual prevention of blinking (77); and orchiectomy that depletes androgens, which are required for tear production and for the normal structure and function of the corneal epithelium (78). Below, we highlight some of the most commonly employed models.

Atropine model. Instillation of 1% atropine sulfate three times per day for five days is reported to rapidly produce typical dry eye manifestations including reduced tear production and abnormal fluorescein staining of the ocular surface. The atropine model is useful mainly to initially assess the protective activity and the ocular pharmacological profile of tear substitutes or ocular drugs (74,79-81). Our own experience with this model has been mixed, and we did not employ it for its lack of reproducibility.

Benzalkonium model. Topical administration of the ocular preservative benzalkonium chloride twice per day for 14 days (75) or three times per day for 4 weeks reported later (82,83) leads to DED. In particular, the experimental rabbits show corneal and conjunctiva damage, decreased aqueous tear basal secretion, loss of goblet cells, and deficiency of mucin-5 subtype AC (MUC5AC) (75).

Autoimmune dacryoadenitis model. This rabbit model, which resembles Sjögren syndrome, was developed by Gou *et al* (69) by co-culturing peripheral blood lymphocytes with purified acinar cells obtained from an autologous LG and injecting the activated lymphocytes into the contralateral gland. The injected LG shows an infiltrate dominated by

Table I. Animal models of DED.

A, Mouse model

Model type	Aqueous-deficient	Evaporative	Mixed
MRL/lpr mouse	Yes	No	No
NoD mouse	Yes	No	No
Lacrimal gland excision model	Yes	No	No
Desiccating environmental stress model	No	Yes	No
Models based on the aging of mice	No	No	Yes

B, Rat model

Model type	Aqueous-deficient	Evaporative	Mixed
Scopolamine model	Yes	No	No
Visual display terminal user model	No	Yes	No

C, Rabbit model

Model type	Aqueous-deficient	Evaporative	Mixed
Atropine model	Yes	No	No
Benzalkonium model	Yes	No	No
Autoimmune dacryoadenitis model	Yes	No	No
Main lacrimal gland ablation model	Yes	No	No
Complete dacryoadenectomy model	Yes	No	No
Concanavalin A-induced model	Yes	No	No
Acute desiccative stress model	No	Yes	No
Closure of Meibomian gland orifices	No	Yes	No
Evaporative DED model (*M. tuberculosis*)	No	Yes	No

D, Cat model

Model type	Aqueous-deficient	Evaporative	Mixed
Main lacrimal gland ablation model	Yes	No	No

E, Dog model

Model type	Aqueous-deficient	Evaporative	Mixed
Spontaneous DED model	Yes	No	No
Main lacrimal gland ablation model	Yes	No	No

F, Non-human primate

Model type	Aqueous-deficient	Evaporative	Mixed
Lacrimal gland excision model	Yes	No	No

DED, dry eye disease; NOD, non-obese diabetic.

CD4+T cells. The ensuing dacryoadenitis leads to reduced tear production, tear film instability and increased ocular surface staining reflecting disruptions in the ocular epithelium (7,69,84).

Main lacrimal gland ablation model. Initially developed to study autologous submandibular gland transfer for treating severe DED in this model, the bilateral lacrimal and Harderian glands and nictitating membrane were removed surgically. Because the aqueous phase of the mammalian tear film is produced by the combined activity of the main and accessory LGs, their removal reduces the total tear volume and protein content (66). However, another paper showed significant dry eye phenotypes associated with elevated ocular surface inflammation observed at 1 month. However, the tear production was not reduced and dry eye phenotypes and ocular surface inflammation gradually improved over a period of 4 months without any additional intervention (66,85,86). To induce severe DED, Li *et al* surgically removed the LG, Harderian gland and nictitating membrane, combined with burning the bulbar conjunctiva with 50% trichloroacetic acid (87). Similar models were also published, like one reporting closure of the LG excretory duct while removing both the nictitating membrane and Harderian gland (67,88).

Complete dacryoadenectomy model. In rabbit models of partial dacryoadenectomy, the removal of only the inferior lacrimal gland (ILG) (without the removal of the superior lacrimal gland) causes partial suppression of tear production leading to inconsistent results (66,67,85,87,89). In view of these limitations, we developed a practical method to completely remove the entire LG of the rabbit as we have described previously (90).

In brief, following the removal of the nictitating membrane, the orbital portion of the superior lacrimal gland was removed through a transcranial approach on the top of the skull; the palpebral portion of the superior lacrimal gland was removed through a transconjunctival approach after the upper eyelid was everted; and finally, the ILG was removed through a transcutaneous approach below the lower eyelid. Determination of TBUT, STT, tear osmolarity, and rose bengal staining of ocular surface showed that the DED induced by complete dacryoadenectomy was stable, chronic, and predominantly aqueous-deficient, thus recapitulating key clinical and histological features of human DED.

Concanavalin A-induced model. Nagelhout *et al* first reported a rabbit model of LG inflammation induced by a single injection of the T-cell mitogen Concanavalin A (ConA) into inferior LGs bilaterally (70). ConA injection elicits a heterophilic infiltrate, which causes severe and widespread tissue destruction. The consequent impaired tear production results in local inflammation of the ocular surface and is associated in measurable changes in aqueous-deficient dry eye clinical parameters.

While trying to reproduce this promising model, we observed that induction of DED by ConA was inconsistent. Exploration of this lack of reproducibility revealed that the blind transdermal injections of ConA into the ILG failed about 40% of the time to reach the LG. This was due to the varied anatomical location of the gland and its 4-fold variation in size. Additionally, the reduced function of the ILG following injection of ConA was compensated for by overproduction of tears by the superior lacrimal gland (71).

These observations led us to improve this model in a way that eliminated such variability. Specifically, we introduced ultrasound-guided injection of the ILG and added injection of ConA to both lobes of the superior lacrimal gland. The results were gratifying.

Briefly, under ultrasound guidance, all periorbital LGs of the rabbit receive one ConA injection; they include the ILG, the palpebral portion of the superior lacrimal gland, and the orbital portion of the superior lacrimal gland. It is critical that the success of the injection into the ILG be confirmed by a post-injection sonogram. A single injection causes acute DED, which lasts for about 1 week. To induce chronic DED, akin to that encountered clinically, ConA injections should be repeated weekly at least two more times for a total of three weeks. Additional injections lead to severe DED reflecting the almost fibrotic status of these glands with commensurate loss of tear-producing parenchyma (71,91).

Evaporative DED model (77,92,93). Miyake *et al* developed a rabbit model of evaporative DED (92) by injecting into the margins of the upper eyelid heat-inactivated *Mycobacterium tuberculosis* dissolved in complete Freud's adjuvant. Three injections are made one each into the nasal, center, and temporal sections of the eyelid. The result is Meibomian gland dysfunction leading to DED in about two weeks.

Acute desiccative stress model. Fujihara *et al* developed this model by keeping the rabbit eye open for 1-3 h using an eyelid speculum in controlled temperature and humidity (24˚C, 55% relative humidity) (77). The corneal damage can be severe, depending on the duration of the desiccative stress. The model has been used to screen drug effects or to study the contribution of desiccative stress to the pathophysiology of DED (63,94).

Closure of Meibomian gland orifices. The Meibomian glands provide most of the polar lipids that cover the tear film. Thus, removing the tear lipid layer promotes evaporation of tear water, a key event in evaporative DED. Gilbard *et al* (76) developed a rabbit model of DED by closing the orifices of the Meibomian glands. To achieve this, they cauterized each of these orifices in the upper and lower eyelids. The result was elevated tear osmolarity, decreased conjunctival goblet cells and corneal glycogen, and reduced corneal wetting (76,95).

Other rabbit models of Meibomian gland dysfunction that feature hyperkeratinized epithelium of the ductal orifices have been produced by topical application of epinephrine or by the systemic administration of 13-cis-retinonic acid (isotretinoin) (96-100). Similar to the rat, DED can be induced in the rabbit by surgical castration or ovariectomy; by injecting botulinum toxin A to the palpebral portion of the superior LG; and by LG denervation (72,73,101,102).

Based on the above, the rabbit appears to be one of the most suitable animals for DED studies. It offers the opportunity to study multiple pathophysiological mechanisms of aqueous-deficient DED in a manner that gives experimental flexibility. For example, the role of the lacrimal gland in DED can be examined from different angles, such as pharmacological intervention, immune damage or complete removal of the glands when tear production is assumed by the accessory lacrimal glands. In addition, the evaporative subtype can be studied probably better than in any other animal (see Table I).

The major drawback of rabbits is the paucity of analytical tools for the detection of proteins in the ocular surface. High-throughput transcriptomic methods offer only a partial compensation for two reasons. Changes in mRNA levels do not necessarily equate changes in the corresponding protein levels, and about one third of the rabbit genome is not yet annotated. In his context, murine models can play a complementary role, especially when genetically modified models are available that can help assess the role of specific pathways in DED.

5. Cat models of DED

Cat models have had limited use in the study of DED. In cats, the removal of the main LG decreased basal tear production as measured by the Schirmer test, but it failed to significantly change ocular surface signs (103). However, McLaughlin *et al* reported that surgical removal of the LG and third eyelid glands resulted in decreased tear production and clinical signs of DED including abnormal corneal fluorescein staining scores (104,105).

6. Dog models of DED

Considering their cost and the demanding effort of operating on dogs, there are only two dog models of DED, only one of which is the result of surgical intervention (ablation of lacrimal glands).

Spontaneous model of DED. Quimby first recognized in 1979 the similarities between Sjogren's syndrome in humans and severe keratoconjunctivitis sicca (KCS) in dogs displaying xerostomia, vaginal dryness, and having multiple serum antibodies (106). Subsequent studies have confirmed these observations (107,108) and demonstrated decreased apoptosis of the lymphocytes infiltrating the LG and increased apoptosis in lacrimal acinar and conjunctival epithelial cells. The American cocker spaniel is the breed with the highest relative prevalence of KCS (20.6%), followed by Lhasa Apso (12.7%) and Shih Tzu (11.5%). However, the commonly used laboratory dogs such as mixed-breed and beagles dogs have lower rates (109). The spontaneous canine dry eye model has been widely used to develop therapeutic interventions for both veterinary and human populations, exemplified by trials of topical application of cyclosporin A (103,110).

Main lacrimal gland ablation model. Bilateral removal of the orbital and the nictitans LGs in dogs induced KCS after 2 weeks, which lasted 6 weeks post-surgery. Tear production was reduced, and the characteristic clinical features of conjunctival hyperemia and accumulation of tenacious discharge were present (111,112).

7. Nonhuman primate models of DED

Among DED models, the monkey model is the one most similar to human DED. Monkeys have one main LG with an anatomical structure similar to that of humans (7). Notably, unlike other species, humans and nonhuman primates do not have a nictitating membrane. Despite these similarities, it has been challenging to develop satisfactory monkey models of DED.

Removal of the LG decreased tear secretion but was not accompanied by reproducible ocular surface damages (103,113). Qin *et al* developed a monkey dry eye model by complete removal of the principal LG and application of 50% trichloroacetic acid to the conjunctiva. These interventions decreased tear secretion, induced loss of goblet cells and infiltration by inflammatory cells within the ocular surface (114).

8. Conclusions

As already alluded to, it is utopian to expect the development of the ideal animal model of DED. This fundamental consideration notwithstanding, the existing models underscore three facts: First, no perfect or nearly perfect model exists. Second, each of the available models captures specific aspects of DED, reflecting the marked heterogeneity of this disease. And, third, existing models, limited as they may be, have greatly contributed to our progress in understanding and treating DED.

The choice of an animal model is important, because there are notable differences in the anatomical, biochemical, physiological, and morphological characteristics of the ocular surface between monkey, dog, rabbit, rat, cat, mouse, and humans. Four features determine the choice of a given model: The size of the eye, its biochemical composition, the available research reagents, and cost. The *size of the eye* can be a major factor for certain experimental needs. In general, the closer in size of the eye of the test animal to the human eye, the more useful this model will be. Smaller eyes can be technically difficult to dissect and obtain ocular tissues of sufficient size to evaluate their response to an intervention or to assay drug levels. In some instances, the size of some ocular tissues is so small that it may require analytical methods of unusually high sensitivity. It is often forgotten that, regardless of species, the eye is one of the smallest and structurally more complex organs.

The *biochemical profile* of the eye varies between species, and this can be in some cases a deciding factor in choosing a particular animal model. An instructive example is the expression of drug metabolizing enzymes such as esterases in ocular tissues. For drugs susceptible to esterase-catalyzed hydrolysis, rabbits and monkeys are closer to the human than mice and rabbits (115).

The *availability of species-specific reagents* weighs heavily in the choice of an animal model of DED. Mice have been the species of choice for modern molecular biology and, as a result, an abundance of reagents and research kits are commercially available for murine proteins compared to other species. In addition, the murine genome has a more robust database than, for example, the rabbit's. Similarly, mice are over-represented amongst the genetically engineered animal models.

Finally, *cost* can at times be prohibitive for small labs, the overwhelming majority of the global biomedical enterprise. For example, the eyes of non-human primates are in many aspects the closest to the human. However, such animals are expensive to acquire and house, and require dedicated personnel for their care and a sophisticated infrastructure to maintain them.

The continuous improvement of animal models for DED is limited by the functional and anatomical complexity of the lacrimal functional unit and by our still limited understanding of DED. The former makes it at times difficult to decipher the contribution of a specific component of this unit to DED. The dacryoadenectomy models presented earlier exemplify this limitation. Even when the periorbital lacrimal glands are removed or surgically denervated, there is residual tear production, whose origin remains uncertain. In reality, DED is not a single entity and, at the very least, its pathophysiology is diverse and perhaps only partially known. Thus, only models addressing specific subtypes of DED have been developed.

The landscape of DED animal models is evolving at a robust pace. Many groups have generated important results using currently available models. The strongest testament to their value is perhaps the many therapeutic agents, approved or under development, against DED whose development depended heavily on animal models. Nonetheless, the complexity of DED and the demands of modern pharmacology require more refined models to address difficult questions such as the identification of dominant mechanistic players or drug molecular targets. Given the talent and dedication of the worldwide ocular research community, continual improvements of these models are likely to occur.

Acknowledgements

The authors would like to acknowledge Dr Ernest Natke (Stony Brook University, Stony Brook, USA) for critically reading the manuscript.

Funding

The present study was supported in part by the National Institutes of Health (grant no. R44EY031193).

Availability of data and materials

Not applicable.

Authors' contributions

WH wrote most of the initial draft with BR, and contributed to revisions. KT reviewed the background literature and contributed to revisions. HP read the manuscript critically and provided general comments. RAH participated in writing and revisions. BR provided general guidance, wrote a number of sections, participated in revisions and finalized the manuscript. All authors contributed to writing and/or critically revising this review. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

BR has an equity position in Medicon Pharmaceuticals, Inc., and Apis Therapeutics, LLC.

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