An Eye on the Dog as the Scientist's Best Friend for Translational Research in Ophthalmology: Focus on the Ocular Surface

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Abstract

Preclinical animal studies provide valuable opportunities to better understand human diseases and contribute to major advances in medicine. This review provides a comprehensive overview of ocular parameters in humans and selected animals, with a focus on the ocular surface, detailing species differences in ocular surface anatomy, physiology, tear film dynamics and tear film composition. We describe major pitfalls that tremendously limit the translational potential of traditional laboratory animals (*ie.,* rabbits, mice and rats) in ophthalmic research, and highlight the benefits of integrating companion dogs with clinical analogues to human diseases into preclinical pharmacology studies.

This One Health approach can help accelerate and improve the framework in which ophthalmic research is translated to the human clinic. Studies can be conducted in canine subjects with naturally occurring or non-invasively induced ocular surface disorders (*eg.*, dry eye disease, conjunctivitis), reviewed herein, and tear fluid can be easily retrieved from canine eyes for various bioanalytical purposes. In this review, we discuss common tear collection methods, including capillary tubes and Schirmer tear strips, and provide guidelines for tear sampling and extraction to improve the reliability of analyte quantification (drugs, proteins, others).

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1.   Introduction

Preclinical animal models provide critical information to better understand human diseases' characteristics, identify biomarkers, develop diagnostic tools and novel therapeutics. Rabbits and laboratory rodents (mice, rats) are widely used for ophthalmic research as they are economical and easy to handle;[1] however, serious drawbacks limit the translational usefulness of data obtained in these species, notably due to the need to artificially induce pathology in these animals (*eg.*, through genetic manipulation or experimental surgery), as well as apparent differences in ocular anatomy and physiology compared to humans. For instance, precorneal residence time of topically applied solutions is much prolonged in rabbits owing to their low blink rate, resulting in 3-fold overestimation of ocular drug exposure if findings were directly extrapolated from rabbits to humans.[2] Another example is topical nepafenac, a potent nonsteroidal anti-inflammatory drug (NSAID) that reaches therapeutic levels in the posterior segment of mice (owing to their thin cornea and small globe size), inhibiting choroidal neovascularization by decreasing production of VEGF[3] – in contrast, humans require intravitreal injections of anti-VEGF compounds to achieve the same outcome. Multiple other examples exist in the scientific literature, together participating to the unacceptably low success rate of ophthalmic clinical trials to date, and resulting in substantial economic loss and burden for scientists, consumers, and society overall.[4] In fact, the main cause for clinical trial failure is either lack of safety or efficacy,[5] two components that are supposedly 'validated' in initial preclinical animal studies.

Under the umbrella of the One Health Initiative, a growing number of investigations have integrated companion animals into preclinical studies to complement and expand the knowledge gained from studies in other animal models, accelerate and improve the framework in which research is translated to the human clinic, and ultimately generate discoveries that will benefit the health of humans and animals.[6] Over the last few years, several review articles have highlighted the benefits of using dogs for translational research in oncology,[7] neurology[8] and other biomedical fields,[6] yet such information is not available in ophthalmology.

The present review provides a comprehensive comparison of key ocular parameters in humans, dogs and traditional laboratory animals (*ie.*, rabbits, mice, rats), highlighting selected strengths and important pitfalls that must be addressed when ocular research is conducted in animal models. This review is focusing on the ocular surface, a critical element of vision that includes the secreted tear film, lacrimal gland(s), eyelids, meibomian glands, cornea, conjunctiva, sclera, and nasolacrimal drainage apparatus. The ocular surface dictates the bioavailability of medications administered topically to the eye,[9] and is a common site of pathology in both human and veterinary medicine. Methods of tear fluid collection for bioanalytical purposes are also being discussed, with special consideration on the safety and efficiency of the collection technique at hand. Lastly, this review highlights on spontaneous and experimental ocular surface disorders in dogs, providing a tool for researchers to better model disease pathophysiology in clinical patients suffering from ocular surface disorders.

2.   Comparative anatomy and physiology of the ocular surface

2.1.   <u>Anatomy</u>

The anatomy of the ocular surface is depicted in **Figure 1** for dogs, and its parameters are being summarized in **Table 1** for all species discussed in this review (*ie.,* humans, dogs, rabbits, mice, rats).

Lacrimal glands - Four types of lacrimal glands can be distinguished in mammals: (i) the orbital lacrimal gland (*glandulae lacrimales superior*), located in the dorsolateral orbit just caudal to the orbital rim, with secretory ducts that open into the upper conjunctival fornix (humans, dogs, rabbits); (ii) the gland of the third eyelid, located in the ventromedial orbit at the base of the nictitating membrane with secretory ducts than open into the nictitans' bulbar conjunctiva (dogs); (iii) the infraorbital gland (*glandulae lacrimales inferior*), located either intraorbital and ventromedial to the globe (rabbits) or extraorbital and caudal to the globe (rodents), with a single secretory duct that opens into the lower conjunctival fornix; and (iv) the Harderian gland, or Harder's gland, extending from the base of the third eyelid into the caudal orbit, with secretory ducts opening at the nictitating membrane (rabbits, rodents).[10,11] The histomorphology of lacrimal glands varies with age and sex of the individual.[12] In dogs, an orbital lacrimal gland and gland of the third eyelid contribute to 60-70% and 30-40% of the overall tear secretion, respectively.[13] The morphological and histological features of the canine glands resemble the human lacrimal gland including distinct lobules and acini that provide serous and mucous secretions, as well as intralobular ducts that drain into small excretory tubules.[14,15] Likewise, an Harderian gland is not present in the canine or human orbit.[14-16] However, two notable differences exist between species: (i) the combined volume of the two canine glands is smaller than the main lacrimal gland in humans (0.24 vs. 0.60 cm^2)[17,18]; and (ii) the accessory lacrimal glands of Krause and Wolfring are absent in dogs (or not yet reported), presumably being consolidated through evolution into the single gland of the third eyelid.[19] These accessory glands account for 10% of the total lacrimal secretory mass in humans but their contribution to the overall tear secretion is negligible (1-2%).[20,21] In rabbits, the histoarchitecture of the main lacrimal gland is comparable to humans with loosely packed acini and round/oval lumen; in contrast, mice and rats have densely packed acini with small pleiomorphic lumen and numerous intercellular tight junctions.[22] Like humans, rabbits also possess accessory lacrimal glands of Wolfring in the tarsal portion of the palpebral conjunctiva.[23] However, the Harderian gland present in rabbits and rodents is a unique anatomical feature that has important repercussions for comparative studies; in fact, the gland's lipid secretions in the tear film have profound effects on the ocular surface physiology (*eg.*, tear composition, tear film dynamics, blink rate) and pharmacology of topically applied medications (see sections 2.2 and 2.3).

Nasolacrimal apparatus - The morphology of the canine lacrimal drainage system is remarkably similar to that of humans, except for a longer nasolacrimal duct (notably in long-nosed dogs), and the presence of accessory duct openings into the nasal cavity.[24] In both species, tear drainage begins with the lower and upper nasolacrimal puncta and canaliculi in the medial canthus, joining into a lacrimal sac in the bony lacrimal fossa, and extending into the nasolacrimal duct that runs through an osseous channel towards the nasal cavity.[25] Species similarities are also evident on a microscopic level, including an epithelial lining with microvilli and mucin-secreting goblet cells, sub-epithelial seromucous glands, and mucosal-associated lymphoid tissue.[24] In contrast, the nasolacrimal apparatus of rabbits has distinct differences compared to humans. Rabbits only have a single nasolacrimal punctum/canaliculus (medial lower eyelid) and the nasolacrimal duct has two very distinct flexures due to the ventral deflection of the snout, a unique feature that results in a convoluted path for tear drainage.[25,26] The fetal development of the rabbit's nasolacrimal apparatus is also unique in mammals, more closely resembling reptiles vs. humans.[27] At an ultrastructural level, the epithelium lining the duct is double-layered (similar to humans) but there are no goblet cells or subepithelial seromucous glands.[28] Nonetheless, the use of the rabbit is still recommended as a practical model to characterize the nasolacrimal apparatus,[29] albeit this choice is described as 'less than ideal' by the

authors. Mice and rats have a well-developed nasolacrimal apparatus that shares similar ontogenetic origin to humans,[27] although the histological features are different. The duct lining is covered by a multi-layered stratified squamous epithelium with goblet cells but without sub-epithelial seromucous glands.[25]

Third eyelid - The nictitating membrane (third eyelid) is a large fold of the conjunctiva that protrudes from the medial canthus over the anterior surface of the globe in many animals, including dogs, rabbits and rodents. The counterpart in humans is the plica semilunaris, a vestigial remnant in the form of a crescent-like conjunctival fold in the medial canthus.[11,30] Despite gross differences, both structures have important physio-morphological similarities such as the presence of goblet cells and lymphoid follicles, contributing to the lubrication and immune protection of the ocular surface.[30] Nonetheless, the presence of a third eyelid should be considered in comparative studies as it could impact ocular examinations (*eg.*, third eyelid protrusion from ocular irritation) or ocular drug delivery (*eg.*, altered retention time of a contact lens),[31] among others. If required for ease of experimentation, a simple fixation of the nictitating membrane can be performed[31] as an alternative to complete surgical removal,[13,32,33] as the latter negatively impacts ocular surface homeostasis.[13,34]

Eyelids - Similar to humans, the canine upper and lower eyelids are comprised of an outer dermis, tarsus, orbicularis oculi muscle, palpebral conjunctiva and secretory tissues including meibomian glands (20-40 per eyelid), glands of Zeis and Moll.[35,36] The main anatomical difference is the tarsal plate, which is comprised of dense fibrous tissue and cartilage-specific components in humans[37] – providing a rigid internal support to the eyelids – compared to a much thinner and poorly-developed fibrous tissue in dogs.[38] Also, the interpalpebral fissure area is approximately 20% larger in dogs (2.2 *vs.* 1.8 cm^2)[39,40], although the measurements of the palpebral fissure width depend on the dog's size and body weight.[40]
The palpebral opening in the rabbit is relatively small (10-16 mm),[38,41,42] albeit much larger than mice (3.7-5 mm)[43] and rats (6-9 mm)[44,45], with a shorter and thicker upper eyelid compared to the inferior palpebrae; consequently, the interpalpebral fissure area is 20% smaller in rabbits than in man (1.44 vs. 1.8 cm^2).[39] The meibomian gland ducts and acini are also larger in rabbits than mice and rats,[46] but the overall volume and distribution of meibomian glands is different than in humans: the total meibomian gland volume in the human (39.5 mm^3) is twice that of the rabbit (18.8 mm^3), with a larger volume in the upper eyelid (man) compared to similar volumes in the upper and lower eyelids (rabbit).[39]

Conjunctiva - The conjunctiva is a thin mucous membrane that serves important roles on the ocular surface including mucin secretion and immune surveillance. The anatomical subdivision of the conjunctiva is the same in humans, dogs, and common laboratory species (rabbits, rodents): the palpebral conjunctiva – lining the inside of the eyelids – reflects back at the level of the conjunctival fornix to form the bulbar conjunctiva, a region that covers the anterior portion of the sclera and attaches to the corneoscleral limbus.[16] However, the amount of bulbar conjunctiva exposed ('scleral show') is notably larger in humans compared to animals given differences in eyelid opening and/or corneal diameter. Another important species difference is the presence of a nictitating membrane in animals (but not man), as the third eyelid is covered by conjunctiva on its anterior and posterior surfaces. As such, animals have two conjunctival fornices in the inferonasal region – one on each side of the third eyelid – and the overall conjunctival surface is generally larger in animals compared to humans. In dogs, the conjunctival area is supposedly larger than in humans given the depth of the canine conjunctival fornices and the amount of conjunctiva covering the canine nictitating membrane,[47] although no objective data exist to date. In rabbits, the upper conjunctival fornix depth (20.36 mm)[48] is larger than in humans (15 mm),[49] while the conjunctival area is reportedly comparable (13.34-

18.48 *vs.* 17.65 cm^2, respectively), although the measurements did not include the rabbit's third eyelid (surgically removed by investigators).[50]

Conjunctival goblet cells are distributed individually in humans, dogs and rabbits, in contrast to clustered organization in mice and rats.[51,52] The distribution of goblet cells is overall similar in dogs and humans, with high density in the canine third eyelid and human plica semilunaris, relatively high density in the conjunctival fornices and palpebral conjunctiva, and lower density in the bulbar conjunctiva.[30,52-56] In rabbits, the highest density is noted at the lid margin of both upper and lower palpebral conjunctivae,[57,58] while the density in the bulbar conjunctiva is generally higher than in humans (399-1576 cells/mm^2 *vs.* 7-979 cells/mm^2).[56,59] In addition to mucin-secreting goblet cells, the conjunctiva also contains an organized immune network termed conjunctiva-associated lymphoid tissue (CALT), a structure that plays a key role in protecting the ocular surface by initiating and regulating immune responses.[60] The presence of lymphoid follicles was confirmed in the conjunctiva of most mammals studied by Chodosh and colleagues – including humans, dogs, rabbits – with the exception of mice and rats,[61] although a later report detected lymphoid tissue in the nictitating membrane of BALB/c mice.[62] At an ultrastructural level, specialized M cells are present in the epithelium overlying the conjunctival follicles in dogs[63] and rabbits,[64] similar to humans.[65]

Cornea - The anatomy of the cornea is unique to each species with important differences in corneal dimensions and ultrastructural features (*eg.*, thickness, collagen arrangement, nerve supply).[11,38,50,66-70] First, the cornea is generally larger in dogs and rabbits compared to humans, while the dimensions are much smaller in mice and rats.[41,44,71-77] As such, the relative amount of cornea and conjunctiva exposed on the ocular surface varies among species, an anatomical fact that has important implications in ocular pharmacology and other research fields; for instance, the surface area ratio of conjunctiva to cornea is two times smaller in rabbits (8.6-8.9) than humans (17.1), a finding that could largely explain species differences in drug penetration into the anterior chamber.[50] Second, the corneal thickness varies among mammals and is generally correlated to the size of the animal.[68] From highest to lowest, the mean central corneal thickness is 497-594 µm in dogs,[68,78] 505-563 µm in humans,[79] 354-407 µm in rabbits,[41,79,80] 159-170 µm in rats[68,80] and 90-137 µm in mice.[68,74,80] The average canine cornea is only slightly thicker than in humans. In contrast, the thinner cornea in rabbits and rodents can limit the use of these laboratory species for selected experiments; for instance, cross-linking is discouraged in corneas thinner than 400 µm due to potential damage to the corneal endothelium or intraocular tissues.[81]

On a structural level, the main layers of the cornea are the same in humans and animals (epithelium, stroma, Descemet membrane, endothelium) with the notable exception of the Bowman's membrane.[11,70] Bowman's membrane is present in nearly all primates (including humans) and selected animals (*eg.*, sheep, deer, giraffe),[82] but is absent in dogs and common laboratory species.[68,70,82] The number of layers and overall thickness of the corneal epithelium vary among species: humans (5-7 layers, 44-55 µm),[79,83] dogs (6-9 layers, 52-64 µm),[70,71,78] rabbits (5-7 layers, 45-49 µm),[38,79,83] rats (10-14 layers, 26-33 µm),[84,85] and mice (13 layers, 37-46 µm).[74] The corneal stroma, comprising nearly 90% of the total corneal thickness in most mammals, is primarily composed of collagen fibrils arranged in lamellae. While extensive collagen intertwining is noted in the majority of the corneal stroma in humans, it is only present in the anterior most-aspect of the cornea in dogs and rabbits.[86,87] Differences in collagen intertwining, along with the absence of Bowman's membrane in laboratory species, explain the vast disparity in stiffness of the anterior stroma (16.2, 1.3 and 1.1 kPa) and posterior stroma (2.5, 0.5 and 0.4 kPa) in humans, dogs and rabbits, respectively.[86,87] The elastic modulus of the cornea is reportedly higher in rodents, although the methodology used was different.[88,89] Corneal rigidity should be considered in comparative studies in which

the biophysical attributes of the cornea are important (*eg.*, wound healing, keratoprosthesis). The corneal endothelium shares a similar morphological blueprint among species (single cell layer, honey-comb pattern), while the cellular density varies from 3233 cells/mm^2 in rabbits, 2875 cells/mm^2 in mice, 2818 cells/mm^2 in dogs, 2732 cells/mm^2 in humans, and 2242 cells/mm^2 in rats.[68,90]

The mammal cornea is the most densely innervated tissue in the body. Corneal nerves play important roles to maintain ocular surface health and homeostasis, including sensory functions (touch, pain, temperature), release of trophic neuropeptides, maintenance of the limbal stem cell niche, and activation of brainstem circuits to promote reflex blinking and lacrimation. From highest to lowest, the sensitivity of the cornea to mechanical stimulus is as follows: humans (0.2-1.0 g/mm^2), rats (0.42-0.47 g/mm^2), mice (0.59 g/mm^2), dogs (2.16-2.9 g/mm^2), and rabbits (6.21-10 g/mm^2).[2,91-95] The murine model is the most extensively studied of all laboratory species given gross similarities between mice and humans in corneal sensitivity and nerve architecture.[67,96] The canine model is also studied in detail given shared features with humans in several spontaneous diseases such as diabetes mellitus, herpetic keratitis, and non-healing corneal ulcers;[97-100] importantly, investigators should account for the canine breed selected for the experiment as corneal sensitivity depends on the dog's cephalic conformation.[95] In regard to rabbits, two striking species differences exist: *(i)* Corneal sensitivity in rabbits is much lower than in humans, dogs and rodents;[2,92] and *(ii)* Morphology of the rabbit subbasal plexus is unique, with nerve fibers sweeping horizontally across the corneal surface in a temporal-to-nasal direction compared to a typical whorl-like or spiraling pattern in other species.[67]

Sclera - Humans have a widely exposed white sclera, a feature that is unique when compared to other primate species (Kobayashi, 1997). In contrast, the scleral exposure is minimal in dogs and routine laboratory species. The thickness of the sclera also differ among species: at the ocular surface (limbal sclera), recorded measurements vary from 0.8 mm in dogs,[101] 0.5 mm in humans,[102] 0.29 mm in rabbits,[103] 0.1 mm or less in rats,[104] and 0.05-0.06 mm in mice.[105]

2.2.   Tear film dynamics

Effective tear dynamics, combined with well-balanced composition of the tear film (discussed in the next section), are critical for the maintenance of ocular surface homeosostasis and physiology. Tear fluid dynamics – or the balance between tear secretion, distribution, absorption, evaporation, and drainage – are closely regulated by the *lacrimal functional unit*. The lacrimal functional unit is unique to each species (see aforementioned anatomical differences), comprised of secreting glands (orbital, accessory, third eyelid, Harder's, meibomian), eyelids, conjunctival goblet cells, corneo-conjunctival surface, and their interconnecting innervation.[106] Key physiological parameters provide insight into the complex tear dynamics – highlighted in **Figure 2** and **Table 2** – and are therefore important to account for in translational studies that involve the ocular surface:

- ***Basal tear turnover rate***: Tear turnover rate is considered a global measure of the tear dynamics and integrity of the lacrimal functional unit.[107,108] The basal tear turnover rate is reportedly 13.1-17.5 %/min in humans,[109,110] 12.1 %/min in dogs,[111] 6.2-7.1 %/min in rabbits[112] and 5.2 %/min in mice;[113] no information was available in rats. In other words, it takes approximately the same time for the tear film to replenish in dogs and humans (~6-8 min) but the duration is longer in rabbits (~14-16 min) and mice (~20 min). The slow tear turnover of rabbits and rodents has important repercussions in translational

research, including a longer precorneal retention time of instilled eyedrops (see next subsection), or exaggeration of ocular surface disease due to delayed clearance of inflammatory mediators from the tear film.[114]

- **Tear volume**: The volume of tears on the ocular surface is highest in dogs (65.3 µL),[111] followed by humans (7-12.4 µL),[109,115] rabbits (1.9-7.5 µL)[112,116] rats (4.6 µL)[117] and mice (0.06-0.2 µL).[113,118] Canine tear volume depends on the subject's body weight but not the dog's cephalic conformation.[111] Differences in study methodology notwithstanding, the canine tear volume is approximately 5 to 9-fold larger than in humans. This discrepancy can be partly explained by the additional secretory tissue in dogs (third eyelid gland) and the larger corneal surface to lubricate in dogs (1.2-2.1 cm^2 *vs.* 1.04-1.3 cm^2).[50,71,77] The canine tear film may also be thicker than in humans (15.1 µm vs. 2.3-11.5 µm), although measurements of tear thickness were only obtained in 6 dogs[111] and the calculation of tear thickness is reportedly highly variable within and between species.[119]

- **Spontaneous blink rate**: The blink action distributes fresh tears on the ocular surface in a uniform layer, promotes secretion of tears from the accessory tear glands, and pumps excess tears (or instilled drop) into the nasolacrimal drainage system. Spontaneous blinking is triggered by higher centers in response to corneo-conjunctival nerve stimulation, presumably due to changes in ocular surface temperature that result from thinning and evaporation of unstable tear film.[120] The spontaneous blink rate is very similar between dogs (14.2 blinks/min)[121] and humans (8.5-17.6 blinks/min),[122-124] although it is lower in rodents (< 5.3 blinks/min)[91,124-126] and *much* lower in rabbits (0.05-0.19 blinks/min).[2,123,127] In other words, *humans and dogs blink approximately every 4-7 seconds*, while *mice/rats blink every 11 seconds* (or more) and *rabbits only blink every 313-1200 seconds*. This large disparity in mammals' blink rate can be explained by species differences in (*i*) ocular surface sensitivity, (*ii*) tear composition, and (*iii*) the inherent stability of the animal's tear fluid. In fact, (*i*) the corneo-conjunctival sensitivity is higher in humans > rodents > dogs >> rabbits, a key parameter that is linked to spontaneous blinking as well as reflex secretion of tear components from the lacrimal glands, conjunctival goblet cells and meibomian glands;[128] (*ii*) tear composition is unique to each species (see next section), for instance large discrepancies exist in the tear lipidomic profile of rabbits *vs.* man;[129] and (*iii*) tear film stability is strongly associated with the maximum blink interval, as recently shown in humans.[130] Tear stability is often measured with the tear film breakup time (TFBUT), defined as the interval between the last complete blink and the first appearance of a dry spot in the tear film. Results of TFBUT and other tear film diagnostics are summarized in **Table 2**, with care given to discard or highlight values obtained in anesthetized or sedated animals (*eg.*, TFBUT of 29.8 min in sedated rabbits)[131] as chemical intervention negatively impacts ocular surface homeostasis (*ie.*, abolished blinking, reduced tear secretion).

Importantly, investigators should account for additional parameters (and their species differences) in any study that involves topical drug administration. In fact, an eyedrop can be considered as a transient ocular irritant – especially if the solution's pH or osmolarity is different than the tear film – thereby stimulating reflex blinking and lacrimation upon contact with the ocular surface.[132]

- **Reflex blinking** (or lack thereof): In dogs, a blink occurs immediately after eyedrop administration and is responsible for removal of any excess solution onto the periocular skin and nasolacrimal drainage system.[40] The same is true in humans, in whom an instilled eyedrop is partially lost (20-30%) due to

reflex blinking and spillage onto the eyelids and eyelashes.[133] Blinking in response to eyedrop instillation is also reported in mice[134] and rats.[135] In contrast, rabbits rarely blink following eyedrop administration, or do so infrequently. In one study, rabbits did not blink for 20-30 minutes after instillation of an eyedrop, and this alone could result in overestimating ocular drug exposure by 3-fold if findings were to be extrapolated to humans.[2]

- ***Reflex tear turnover rate***: Eyedrop administration abruptly increases the volume of fluid in the conjunctival sac and ocular surface. The sudden disruption in homeostasis promotes a faster nasolacrimal drainage until baseline conditions return. This physiologic response is prominent in dogs (50 %/min)[111] and humans,[109,115] but is minimal in rabbits (6.1-6.9 %/min).[112] In fact, the tear turnover rate in rabbits is mostly unchanged whether a small (1-5 µL) or large volume (25-50 µL) of eyedrop is instilled on the ocular surface,[112] a finding likely related to the poor corneal sensitivity and inexistent/minimal reflex blinking in this species.[2,92] No available report in mice or rats can be found in the literature.

- ***Volumetric capacity of the palpebral fissure***: The surface of the canine eye can 'hold' on average 31.3 µL of fluid,[40] nearly identical to the volumetric capacity of the human eye (25-30 µL)[109,136] and the volume of a single ophthalmic drop (35 µL).[137] Of note, the volumetric capacity of the canine eye is positively correlated with the length of the palpebral fissure,[40] and may be larger in breeds larger than Beagles (*eg.*, German Shepherd dogs).[137] The exact volumetric capacity of the eye is not reported in laboratory species, but is presumably around 10-25 µL in rabbits (based on drug quantification in tears at various instilled volumes),[112,138] $\leq$ 5 µL in mice[139,140] and $\leq$ 20 µL in rats.[141]

2.3.   Tear film composition

The tear film is a complex biological fluid containing thousands of compounds of diverse structures and functions, including proteins, lipids and mucins, as well as minor constituents such as electrolytes, vitamins, and growth factors.[36,142] The integrated interactions of these constituents are responsible for the promotion of a stable tear film and, ultimately, the homeostasis of the ocular surface. Species differences in tear film components are summarized in **Table 3**.

Proteins – The total protein content is generally similar in dogs (5.2-14.6 mg/mL)[143] and humans (6.0-11.0 mg/mL),[144] although qualitative and quantitative differences exist. Specifically, the three major constituents of the human tear proteome (lactoferrin, lysozyme, lipocalin)[144] are only detected at low levels in dogs,[145-150] although the relative abundance of other common proteins (*eg.*, lacritin, secretory IgA, serum albumin) is generally similar between the two species. Importantly, homologous proteins have been described in canine tears and may play similar functions to their human counterparts – for instance, *transferrin* is an iron-binding protein with similarities to lactoferrin, while *major canine allergen* is an abundant protein in canine tears with similarities to lipocalin.[148-150] From a qualitative aspect, a recent in-depth proteomic study showed that 25 out of 125 proteins detected in canine tears were common to humans.[149] In rabbits, Wei et al. found that the total protein content was two-fold higher in rabbits compared with humans (20.6 mg/mL *vs.* 9.4 mg/mL), although the number of different proteins detected in tear samples was lower in rabbits.[151] Other differences in tear proteins among species are summarized in **Table 3**.

Mucins – Ocular mucins are large glycoproteins expressed by conjunctival goblet cells, the corneal epithelium and the lacrimal gland(s), playing important roles on the ocular surface in lubrication, wettability and barrier function.[152] The main secretory mucin, MUC5AC, is described at large levels on the ocular surface of humans and animals.[11,55,152] The expression of membrane-associated mucins, however, differs among species. In a recent study by Leonard et al., dogs were found to have a very similar pattern of mucin expression to that of humans and rhesus macaques, with MUC16 being the most abundant mucin transcript.[153] In contrast, the rabbit had a unique mucin expression pattern with all mucin transcripts expressed at relatively similar levels; as such, the authors concluded that the predictive value of the rabbit as a model in ocular surface studies should be called into question.[153] In another study, the majority of ocular mucins detected in dogs and rabbits were neutral fucosylated glycans, while the ones in humans were mainly negatively charged sialylated glycans;[154] however, the experiment lysed the ocular surface epithelium and could not discriminate between mucins of differing origin.

Lipids – In a comprehensive lipidomic study comparing the meibum collected in several species, Butovich et al. found that the highest degree of biochemical similarity with humans was observed in mice, closely followed by the dog.[129] An earlier study by Butovich et al. also reported the close resemblance of the tear lipid composition between dogs and humans.[155] In these 3 species (humans, dogs, mice), the major lipid classes included wax esters, cholesterol esters, and o-acyl-ω-hydroxy fatty acids (OAHFA). In contrast, the major lipid classes in rabbit tears were DiHL esters (24,25-dihydro--lanosterol esters), diacylated diols, and OAHFA, with low to trace amounts of wax and cholesterol esters.[129] Such discrepancy between rabbits and humans was confirmed in a separate study by Wei et al, who noted significant differences in the tear film concentrations of triglycerides (higher in rabbits), free cholesterol (lower in rabbits), phosphatidylcholine (higher in rabbits) and phosphatidylethanolamine (higher in rabbits).[151] Taken together, the authors of these two studies argued that the rabbit is too different to serve as a valid animal model for humans, at least from a biochemical standpoint.

3.   Tear collection for bioanalytical purposes

The tear film, a complex body fluid uniquely exposed to both internal and external environments, contains numerous endogenous and exogenous molecules (*eg.*, proteins, lipids, mucins, xenobiotic) that can be assayed for clinical or research purposes. Topically and systemically administered drugs can be quantified in tear fluid to determine the clinical efficacy and dosing frequency from fitting of kinetic data.[156-160] Multiple 'omics' approaches can also be utilized for analysis of the tear fluid including proteomics,[149,150,161-166] lipidomics[129,155,167,168] and metabolomics,[168] providing valuable information for the development of novel diagnostics and therapeutics in ophthalmology, as well as biomarkers identification for various ocular and systemic diseases.[169-171] However, collecting tears and obtaining reproducible analytical results in ophthalmology is challenging; in particular, the volume of tear fluid is limited (unlike other biological fluids, such as blood or urine), and the biochemical profile of a tear sample is intimately affected by the collection, storage, extraction, handling, and analytical methods used by the investigator.
In this section, we review the main sampling methods reported in the scientific literature and discuss their respective advantages and limitations. Further, based on the authors' experience with dogs in clinical and research settings (board-certified veterinary ophthalmologist [LS] and pharmacologist [JPM]), the section provides recommendations specific to canine subjects and their use in translational research (**Figure 3**).

3.1. Direct tear sampling

A microcapillary glass tube (1-10 µL) placed in contact with the inferior lacrimal lake is the most commonly reported technique to collect tear fluid. This method directly samples tear fluid by capillary action and is extensively described in humans,[144,161,166,172-185] dogs,[147,150,186-189] rabbits,[183,184,187,190,191] mice,[192,193] and rats.[147,183,194,195] Other direct techniques (seldom reported) involve micropipettes,[196] polypropylene tubing[147] or polytetrafluoroethylene tubing.[172] With capillary glass tubes, it is possible to obtain unaltered tear samples by avoiding reflex tearing from ocular irritation, especially if the collection is performed by an experienced operator on a cooperative patient. The minimal binding of tear compounds to glass is another reported advantage of capillary tubes. However, the main limitation of microcapillary collection is the ***long collection time***, generally ≥ 5 min[174,180,184,185,190,193] – this is particularly true in small laboratory animals, with up to 15-30 minutes and 15-60 min required to collect sufficient tear fluid in rabbits[184,190] and rodents (mice and rats),[193,194] respectively. Another critical limitation of direct sampling is the ***low volume*** of tear fluid retrieved, generally ≤ 5 µL[144,147,166,172,175,177-179,182,183,185,191-193] – as such, the small sample collected may be grossly insufficient in some individuals,[144,188] may require excessive dilution that renders the target analyte undetectable,[189] and does not take into account possible losses (*eg.*, transfer, storage) or the need to repeat certain assays in duplicates.

Several strategies can be used to overcome current obstacles with the volume of the tear volume; however, each come with its own set of drawbacks (listed in parentheses): (*i*) Sedate or anesthetize the animal to extend collection duration and obtain a larger volume (altered lacrimal functional unit and ocular surface homeostasis);[147,184,190,192-195] (*ii*) Pool tear samples from several subjects (reduced statistical power and loss of information regarding inter-individual variability);[161,187,192] (*iii*) Induce reflex tearing with a stimulant – either physical (*eg..* irritation to nasal mucosa or cornea), chemical (*eg.*, parenteral pilocarpine or ammonium fumes) or physiological (*eg.*, yawn or sneeze reflex) – thereby accelerating tear flow and shortening collection time (diluted tear sample, unable to control flow rates);[180,193,194,197] (*iv*) Instill fluid (*eg.*, saline) on the ocular surface immediately prior to tear collection, a process called 'flush' or 'washout' that yields a larger tear sample in a shorter amount of time (diluted tear sample, non-standardized instilled volume, non-homogenous mixing of fluid with tears).[174,180,192,193,195,197,198] In particular, the diluting effect of reflex tearing or flush methods may drop the concentration of low-abundant compounds below the analytical limit of quantification, and potentially mask differences between groups due to reduced variance in tear composition.[174,199] A third limitation of microcapillary tubes is the ***technical difficulty*** associated with the collection method. In fact, it is nearly impossible (or very challenging) to avoid reflex lacrimation in a consistent manner, even with cooperative patients and experienced personnel;[175,176,180,197,200] for instance, capillary tear collection by Markoulli et al. resulted in tear secretion that was approximately 4-fold faster than basal tear flow in humans (4.6 *vs.* 1.2 µL/min, respectively).[109,180] Of note, sampling itself may act as a stimulant due to environmental factors (air movement, light)[200] and the stress/anxiety experienced by patients when capillary tubes are used.[173,176,179,197] Importantly, the technical challenge of capillary tubes is amplified in animals given their uncooperative nature, and in any patient with aqueous tear deficiency given the low tear volume; tear sampling can be extra slow in these cases, possibly impeded/interrupted if an air bubble or mucinous material enters the capillary lumen.[185]

Taken together, although direct tear collection remains the preferred method of some investigators given the 'undisturbed' tear sample retrieved,[199] the serious drawbacks listed above have prompted a growing number of clinicians and researchers to consider indirect tear sampling in humans as suitable alternatives.[149,176] It is the authors' opinion that indirect tear sampling is also preferred in dogs, cats and

laboratory animals. Ultimately, the patient's safety and comfort during tear collection is paramount and, as suggested by Berta, '*it is better to use well-controlled methods than to try to cause as little irritation as possible*'.[200]

3.2.   Indirect tear sampling

Indirect techniques involve tear fluid absorption with either Schirmer tear strips or absorbent sponges, followed by extraction of tear compounds by centrifugation and/or solvent elution.

Schirmer tear strips are routinely used to measure tear volume for clinical assessment of dry eye disease in humans and veterinary species.[165,201-206] The strips are made of Whatman no. 41 cellulose filter paper and possess specific characteristics to promote tolerance (5mm width x 35mm length, 0.22 mm thick, 20-25 µm porosity, foldable extremity for ease of insertion).[197,207] In addition to their conventional use for aqueous tear assessment, Schirmer strips can retrieve tear fluid for bioanalytical purposes in humans,[144,161,165,166,173,176,208] dogs,[143,149,156,159,160,186,209] and small laboratory species.[191,210,211] For instance, Schirmer strips were used for in-depth characterization of proteomics in human[162] and canine tears,[149] and can also successfully recover specific analytes such as cytokines,[208,212] clusterin,[144] and xenobiotics.[156,158-160,191,213]

Absorbent sponges exist in different material types such as cellulose,[179,181,214,215] polyvinyl acetal,[143,159,173,181,191,214,216,217] polyester,[185,188,217] and polyurethane.[181] A material with hydrophilic and hydrophobic properties (*eg.*, polyvinyl acetal, polyurethane)[181,214] is generally preferred in order to optimize the amount of fluid absorbed and the amount of fluid retrieved from the sponge. For tear fluid collection, the sponge is held against the lacrimal lake by the operator (to minimize reflex tearing),[179,185,188,191,217] or placed beneath the lower eyelid for a given period of time.[159,181,214] Tear fluid recovered from absorbent sponges can be assayed for selected tear compounds, similar to Schirmer strips.

Indirect tear collection is superior to direct capillary sampling in many aspects, namely: (i) Improved tolerance and acceptability by patients;[144,176,217,218] (ii) Ease of use and operator safety, especially for Schirmer strips, allowing non-specialists to perform the procedure with minimal training;[176,181,217] and (iii) Larger volume of tears collected in a shorter duration.[179,181,185,191] Absorbent materials collect tears but can also pick up cellular and extracellular 'debris', an attribute considered beneficial by some as the sample obtained is more representative of the dynamic microenvironment at the ocular surface,[219] but also perceived as a limitation by others as the fluid retrieved is not 'pure' tears.[199] On this note, the main limitation of indirect sampling is the 'invasiveness' of the technique, at risk of promoting reflex tearing and altering the composition of the tear fluid; indeed, several studies showed variable tear composition between directly- and indirectly- collected samples, with notable differences in the qualitative and quantitative profiles obtained for tear lipids[167] and tear proteins.[161,166,182,220] Another important drawback is related to the adsorptive properties of Schirmer strips or absorbent sponges, *ie.* incomplete release of tear compounds following extraction;[143,159,207,208,216] however, the authors believe this limitation can be minimized/controlled with adequate precautions (see section 3.3).

3.3.   Proposed strategy for lachrymal determinations in dogs

Schirmer strips vs. absorbent sponges – Sponges can rapidly absorb up to 106 µL of tear fluid in dogs,[214] while the maximum absorptive capacity of Schirmer strips is ~ 31 µL (*ie.*, 35 mm wetness).[221] With sponges, however, the operator can only control the duration of tear collection and not the volume of tears soaked up in each individual. The resulting variability in tear volume absorbed often translates into large intra- and

inter-subject variability in the concentration of the compound(s) of interest, as shown for protein content[143] and various drugs such as doxycycline,[159] minocycline,[222] voriconazole,[223] and ofloxacin.[191] On the other hand, the ability to control the volume of tears absorbed with Schirmer strips (*ie.*, same mm mark) generally improves the reproducibility of the results.[143,159,160,224]

As such, the authors prefer (*i*) absorbent sponges for collecting large volumes of tears in canine subjects – *ie.,* for further use as blank tears in bioanalytical assays for example – and (*ii*) Schirmer strips for collecting known amounts of tears in any scenario where reproducibility of the data is important (*ie.,* for group comparisons, or follow-up of the same individual over time).

Schirmer strips for protein quantification – For consistency purposes, the authors recommend the use of dye-free Schirmer tear strips, being consistent with the manufacturer and lot number (given the reported variability in absorptive and adsorptive properties among Schirmer strips),[225,226] as well as the time of collection (*eg.*, morning)[176], because of known diurnal variability in lacrimal protein composition in humans[182,197] and dogs.[224] The distal end of the Schirmer strips should remain in position (ventrolateral conjunctival fornix) until 20 mm, 25 mm or 30 mm wetness is reached.[160,186,209,221,224] Strip wetness < 20 mm is discouraged given the potential '*concentrating effect*' of the absorbent filter with low tear volumes,[143] while complete wetness of the strip (35 mm) should be avoided as the total protein content is significantly greater with 35 mm compared to 20-30 mm mark,[221] likely due to vascular fragility and excessive irritation ensued by the prolonged test duration. Importantly, investigators should be consistent with the selected mm- mark (strip wetness) within and between patients in order to standardize the volume of tears collected among subjects. This strategy provides a lower coefficient of variability in tear protein content compared to ophthalmic sponges[143] and capillary glass tubes[224] in dogs, thereby improving the reliability and reproducibility of the data. Tear extraction and protein analysis can be done directly after tear collection, or can be postponed to a future date as long as Schirmer strips are stored immediately at -80°C and the stability of the compound(s) of interest is verified.[197] Following tear extraction with centrifugation,[143,176,221,224] elution in solvent,[144,149,161,162,186] or a combination of both,[208,227] total protein content (TPC) should be quantified in order to standardize the amount of sample used for subsequent analyses.[149,162] The authors' preferred method is infrared spectroscopy with Direct Detect™ (EMD Millipore, Danvers, MA) as the technique utilizes merely 2 μL of tear sample, without any of the drawbacks of colorimetric protein assays (*eg.*, Bradford, Lowry), including variability with specific protein composition and potential contamination from the absorbent material.[179,228]

Schirmer strips for drug quantification – The following steps should be considered to optimize drug quantification in pharmacological studies:

- **Study design:** In studies that assess tear film pharmacokinetics following topical drug administration, one must consider a potential limitation associated with Schirmer strips which remove most of the tear fluid in early collection times, thereby negatively impacting the 'true' tear concentration at later time points.[197] For this reason, the authors recommend to conduct pharmacokinetic studies in tears over several days (*eg.*, 10 days for 10 collection time points), repeating topical administration each day with a standardized volume and limiting the collection to a single time point per day. An alternative is to use a larger sample size and randomly allocate each time point to a subset of individuals or eyes (*eg.*, 40 eyes with n = 5 eyes for 8 separate time points),[156] although this method should account for differences between subjects such as greater tear volumes in dogs of larger body weight.[111]

Another aspect to consider in the study design is the assessment of drug kinetics in diseased eyes, rendering the study results more clinically applicable (see section 4.2); in fact, tear film concentrations and ocular bioavailability are likely to differ in healthy *vs.* diseased eyes (*eg.*, excessive lacrimation, increased absorption into congested conjunctival vessels, albumin binding),[160,229] yet the majority of ocular studies to date are conducted in healthy individuals which is a clear limitation for translation of research findings from bench to bedside.

- **Tear collection with Schirmer strips:** It is important to homogenize the volume collected within and between subjects by standardizing the extent of strip wetness (≥ 20 mm mark) – this approach limits the variability in tear concentrations related to the collection method itself.[159,160] The amount of wetness is then converted to a volume (µL) in order to calculate actual tear film concentrations;[221] data reporting is otherwise limited to µg/g of strip.[156,191] In dogs, the median volume absorbed by Schirmer strips is 18 µL (20 mm), 22 µL (25 mm), 26 µL (30 mm) and 31 µL (35 mm), information obtained from hundreds of *in vivo* collections with pre- and post- weighing of Schirmer strips).[221] This method is preferred over *in vitro* use of phosphate buffered saline[208] given differences in fluid viscosities and the inability of an *in vitro* experiment to mimic the complex dynamics of tear absorption noted *in vivo* (*eg.*, rapid initial uptake,[204] tear evaporation).
  In parallel, investigators should record the duration of tear collection (*eg.*, 50 seconds to reach 20 mm) in order to calculate a flow rate (µL/min) for each sample obtained.[159,180] In one of our experiments with doxycycline in dogs, flow rate did not influence tear concentrations,[159] but this finding might not be generalizable to other drugs and/or other species of interest.

- **Extraction protocol optimization:** A drug can be extracted from Schirmer strips via centrifugation, solvent elution, or a combination of both methods. However, a single extraction protocol cannot be generalized to all pharmacological studies as the specific physicochemical properties of each drug (*eg.*, molecular weight, lipophilicity) can affect the extraction efficiency from the filter papers.[207] As such, investigators should consider conducting a preliminary experiment to determine the optimal extraction protocol for the drug studied, and report specific recovery rates (mean $\pm$ standard, range). For instance, the recovery of prednisone and prednisolone was maximized with a combination of centrifugation and elution in methyl tert-butyl ether, a solvent chosen over methanol and acetonitrile based on superior drug extraction from Schirmer strips (**Figure 4**).[160] Of note, a comprehensive review of all reported protocols is beyond the scope of the present work, and further research is warranted to assess the potential benefits (or lack thereof) of extraction steps reported in the literature, such as cutting Schirmer strips into small pieces[162,165,213] or using ultrasonic agitation.[160,165,197] Ultimately, an optimized extraction protocol is important as it enhances the reliability of the data at hand, improving the sensitivity of the bioassay, and providing drug concentrations closer to 'true' biological levels in the tear film.

- **Bioanalytical method optimization:** First, internal standard should be applied directly onto the dry portion of the Schirmer strip (**Figure 4**),[160] *ie.* before tear extraction instead of post-elution with solvent as routinely described;[156,213] this step allows for drug quantification to account for potential variability in extraction efficiency between samples. Second, the standard calibration curve solutions should be constructed by spiking known drug concentrations and internal standard onto Schirmer strips, followed by the same extraction protocol as for biological samples; this step is equivalent to 'spike and recover'

experiments recommended for other analytes such as cytokines[216] and proteins.[207] Third, actual tear fluid should be used whenever possible as the selected matrix for standard calibration curve and quality control solutions,[159,160,223] as the reported surrogates (*eg.*, artificial tear solution)[230] do not account for chemical interferences and matrix effects that typically occur with a complex biological fluid (*eg.*, ionization suppression).[223] Blank tears can be collected with absorbent materials prior to study initiation, retrieving up to 84 µL in 1 min with ophthalmic sponges in dogs[214] and 132 µL in 12 min with successive substitutions of polyurethane mini-sponges in humans.[181]

4. Spontaneous and experimental models of ocular surface disorders in dogs

4.1. <u>Spontaneous ocular surface diseases in dogs with translational applications to humans</u>

Spontaneous ocular surface disorders are common in dogs and represent one of the major causes for referral visits to veterinary practitionners.[231] In contrast, naturally-acquired ocular surface pathology is much less common in rabbits[232,233] and is rare in mice and rats.[234-236]

4.1.1. Keratoconjunctivitis sicca

Keratoconjunctivitis sicca (KCS), or 'dry eye', represents one of the most common ocular diseases in humans with an estimated prevalence ranging from 5 to 50% in different regions worldwide.[237] The disease is also very common in dogs (prevalence 1.5 to 35%),[231] although not a single report of spontaneous KCS case exists in laboratory animals such as rabbits, mice and rats.

The pathogenesis of KCS is very complex, involving diverse physio-anatomical factors such as lacrimal gland integrity, meibomian glands function, hormonal balance and neuronal input.[237] Numerous models of dry eye have been established in animals over the years,[238-240] helping to elucidate complex pathological mechanisms involved in KCS and develop novel therapeutics for humans. However, the major drawbacks of most animal models are the acute nature of the induced pathology (*vs.* chronic disease in humans) and the focus on a single component of the lacrimal functional unit, such as surgically removing the lacrimal gland in mice to reduce tear secretion,[32] cauterizing the lid margin in rats to induce meibomian gland dysfunction,[241] or instilling topical 1% atropine in rabbits to disrupt the efferent neural input.[242] These experimental models can be generally improved by increasing the number of interventions in the study animals, for instance combining lacrimal glands removal with chemical destruction of the conjunctiva in rabbits,[243] or combining scopolamine administration with desiccating environmental stress in mice; yet, these complex models remain suboptimal at best given the acute nature and the inability to fully encompass the complexity of KCS pathophysiology.[244] Dogs, on the other hand, develop KCS in a spontaneous manner and do not require invasive procedures to disrupt the lacrimal functional unit.[201,205] Most importantly, the disease is clinically and immunopathologically similar to dry eye in humans, and possesses several attributes that are beneficial for translational research:

- Canine KCS is typically bilateral, develops in middle-aged animals, is more common in female dogs and in certain breeds (*eg.*, American Cocker spaniel, English Bulldog), mimicking the diversity of dry eye in humans related to sex and race.[201,237]
- Immune-mediated dacryoadenitis is the most common etiology of KCS in dogs – similar to human patients with Sjögren's syndrome – in which progressive lymphocytic infiltration of the lacrimal gland(s) damages the secretory tissues and reduces aqueous tear production.[201]

- Meibomian gland dysfunction is recognized in many canine patients with ocular surface disorders, affecting tear film stability in a similar manner than evaporative dry eye in human patients.[245]
- Spontaneous symptoms of ocular irritation, conjunctival hyperemia and corneal scarring correlate directly with aqueous tear production, a parameter that is easily measured/quantified using a standard Schirmer tear test strip.
- Multiple diagnostic tools used in humans can easily be applied in dogs (but not rodents) given the large size of the canine globe,[205,239] including tear osmometry, vital staining, strip meniscometry test, infrared meibography and corneo-conjunctival impression cytology.
- Dogs and humans display a similar responses to common therapeutics for dry eye disease; in fact, the two FDA approved anti-inflammatory drugs for dry eye disease in humans (cyclosporine, lifitegrast) were first developed in canine patients with spontaneous KCS.[201,246]

The main limitation to consider in dogs is the tendency for clinical signs to be more pronounced in that species vs. humans (*eg.*, tenacious mucoid discharge, corneal melanosis, neovascularization), in part because canine KCS is often diagnosed at a later stage when owners fail to recognize more subtle clinical signs early on.

4.1.2. Allergic conjunctivitis

Allergic conjunctivitis is a common disorder in humans with an approximate prevalence of 40% in the North American population.[247] The disease is characterized by an immunopathological reaction of the ocular surface to the external environment, resulting in clinical symptoms that range from mild conjunctivitis (seasonal or perennial) to the more severe, vision-threatening vernal keratoconjunctivitis and atopic keratoconjunctivitis.[247] Over the past few decades, extensive research on small laboratory species (mice, rats, guinea pigs) has helped elucidate some of the complex molecular and cellular processes involved in the pathogenesis of ocular allergies.[248,249] However, these experiments primarily relied on a relatively small selection of allergens (*eg.*, ovalbumin, compound 48/80, ragweed pollen), using an experimental design that merely mimics acute forms of the disease – not chronic allergen exposure over months to years – therefore limiting the long-term clinical significance of these findings. On the other hand, dogs possess notable benefits for the comparative study of allergic conjunctivitis, especially when considering companion animals rather than laboratory Beagles: (*i*) these animals share the same environment (and related allergens) as their human owners, unlike commonly used species who are housed in a laboratory setting; (*ii*) companion dogs are outbred, providing a genetic diversity background that better reflects the human population than inbred laboratory species; and (*iii*) dogs develop a spontaneous form of allergic conjunctivitis. Spontaneous allergic conjunctivitis is relatively common in dogs, often associated with other allergic disorders such as canine atopic dermatitis.[250] Similar to humans, the clinical signs of allergic conjunctivitis involve conjunctival hyperemia, chemosis, pruritus and ocular discharge, the disease can be diagnosed with high sensitivity and specificity using the conjunctival provocation test,[250] and similar therapeutics are used in both species including topical antihistamines, mast-cell stabilizers, NSAIDs and immunomodulators.[247]

4.1.3. Microbial keratitis

It is well recognized that a natural host is best suited for studying infection, as several species-specific factors (*eg.*, anatomical, physiological, genetic, immune) closely influence the host-pathogen interactions

and subsequent clinical response.[251] These factors likely explain why rabbits and rodents – traditionally used to model ocular surface infections in humans – cannot fully recapitulate the disease presentation and progression that occur in human patients.[252,253] As such, there is an emerging appreciation for the translational advantage of studying spontaneous (and not experimental) ocular infections in dogs:

- **Herpetic keratitis**: Recent work has highlighted the robustness and reproducibility of the canine model to study ocular herpesvirus infections and disease,[253,254] showing striking similarities in the pathogenesis of canine herpesvirus-1 and herpes simplex virus-1, both members of the alphaherpesvirinae subfamily with a seroprevalence of 21-98% in dogs (CHV-1) and 67-90% in humans (HSV-1).[253]
- **Bacterial keratitis**: The most common bacterial genera isolated from canine patients overlap with the ones recognized in human patients (Staphylococcus, Streptococcus, Pseudomonoas).[255,256] In fact, the major culprit in canine bacterial keratitis (Staphylococcus pseudintermedius) is now considered an emerging zoonosis in humans.[257]

4.1.4.   Others

Dogs can serve as models for other ocular surface diseases such as corneal endothelial dystrophy (analogous to Fuch's dystrophy in humans),[258] limbal stem cell deficiency,[259] ocular surface squamous neoplasia[260] and neurotrophic keratopathy,[261] among others.

4.2.   Breakdown of the blood-tear barrier in dogs: A model for ocular pharmacology and therapeutics

4.2.1.   Histamine-induced conjunctivitis

To date, the vast majority of preclinical ocular studies for evaluation of candidate drug efficacy and safety are conducted in healthy eyes – in part for simplicity, but at a higher risk of treatment failure rate when translating these findings to clinical studies. Indeed, healthy eyes do not account for the disruption of ocular homeostasis that occurs with inflammatory diseases, including (but not limited to) changes in tear film dynamics, tear composition and permeability of ocular tissues. To address this shortcoming, the authors have recently established a robust *in vivo* model of conjunctivitis in dogs, a translational large animal model that provides a unique opportunity for scientists to investigate the ocular surface in health and disease states.[212] The model specifically focused on conjunctivitis as this condition is frequently encountered in humans and dogs,[262,263] developing either as a primary condition (*eg.*, bacterial, viral), or as a bystander to common ophthalmic diseases such as blepharitis, keratitis, uveitis and glaucoma. This model is particularly appealing given the low cost, non-invasiveness, self-resolving nature, ability to adjust the duration and severity of the disease, and shared features with naturally occurring diseases in human and veterinary medicine.

The main highlights of the translational 'large animal' model are as follows:

- The selected compound (histamine) is inexpensive and triggers local inflammation in a non-specific manner.

- Disease severity is dose-dependent, allowing investigators to induce mild (1 mg/ml), moderate (10 mg/ml) or severe (375 mg/ml) conjunctivitis (**Figure 5**).
- Disease duration is dose-dependent, self-resolving within an average of 115 min (1 mg/ml), 190 min (10 mg/ml) or 390 min (375 mg/ml). The duration of conjunctivitis can be lengthened by repeating topical histamine administration at set intervals.[160]
- Topical histamine is safe and generally well-tolerated, although selected eyes receiving the highest dose of histamine (375 mg/ml) can develop mild ocular irritation (lasting < 1 min), blepharitis or miosis.
- Tear film composition changes in eyes with experimentally-induced conjunctivitis (*eg.*, higher levels of serum albumin and inflammatory cytokines), mimicking clinical patients with ocular surface inflammation.
- A transient increase in tear quantity and decrease in tear quality occur, although tear film homeostasis is rapidly restored in ≤ 5 min.[264]

Levels of serum albumin are increased in tear film of canine eyes with experimentally-induced or naturally-acquired conjunctivitis,[212,224] a physiological variation caused by the breakdown of the blood-tear barrier (**Figure 6**). Disruption of the blood-tear barrier is also described in human patients with spontaneous ocular surface disorders (*eg.*, dry eye, allergic conjunctivitis)[182,265-267] and other animal species (rabbits, horses, guinea pigs).[268-270] Increased vascular permeability and disruption of tight junctions between conjunctival epithelial cells likely play a role (**Figure 6**),[271,272] although the exact etiopathogenesis is unknown and require further investigation. A few noteworthy limitations are listed here: *(i)* the model is not adequate to study ocular allergy given the lack of characteristic features noted in canine patients with allergies (*eg.,* follicular conjunctivitis); *(ii)* pro-inflammatory mediators other than histamine are also responsible for triggering conjunctival inflammation in clinical patients (*eg.*, leukotrienes, cytokines); *(iii)* conjunctival inflammation is relatively short-lived (115-390 min) and cannot mimic the physiological changes noted in patients with chronic conjunctivitis (*eg.,* reduced goblet cell density).

4.2.2.   *Clinical relevance of serum albumin leakage in tear film*

Elevated serum albumin levels in the tear film represents a biomarker for ocular insult or inflammation in humans, dogs and other species.[182,212,265,270] In brief, plasma-derived albumin leaks onto the ocular surface from congested conjunctival vessels and mixes with the tear film; as such, albumin concentration in tears is generally low in healthy state but increases substantially in diseased eyes.[182] For instance, a recent study showed that canine eyes with diverse ocular diseases (*eg.*, corneal ulcer, uveitis, glaucoma) had lacrimal albumin levels that were up to 14.9-fold greater than contralateral healthy eyes.[212] Albumin is a relatively large protein that has a remarkable capacity for binding ligands.[273] At the level of the eye, protein binding represents an important restriction to drug absorption as only the unbound fraction of the drug diffuses across the ocular tissue barriers.[268] Combined with the rapid drainage of tears following eyedrop administration (in humans/dogs, not true in rabbits), any portion of drug that binds to albumin in tear film can be considered as 'lost' from a pharmacological standpoint. Broader implications of the blood-tear barrier breakdown on ocular drug pharmacokinetics are listed below:

- **Reduced bioavailability for intraocular targets**: The inability of bound therapeutic drugs to penetrate the cornea lowers the amount of drug available inside the eye to exert its pharmacological action. The physiological effects of increased albumin levels in tears was recently demonstrated with tropicamide

(and to a lesser extent latanoprost) in dogs,[229] as well as pilocarpine in rabbits.[268] Of note, the impact of lacrimal albumin on the pharmacological activity of a given drug is likely modulated by various factors, the concentration of the formulation, the mechanism of action and the potency of the drug for its biological target.[229]

- **Reduced bioavailability for ocular surface targets**: Drug-albumin interactions in the tear film could also be detrimental for management of ocular surface disorders, for instance reducing the efficacy of therapeutics for bacterial keratitis as only the unbound portion of an antibiotic is microbiologically active.[274] Preliminary experiments conducted by the authors showed that the presence of albumin results in higher minimal inhibitory concentrations (*ie.*, reduced susceptibility) for various antibiotics against common bacterial isolates in dogs (in-house unpublished data).

- **Tear film concentrations of systemically administered drugs**: Drug in the plasma compartment can access the tear film by active secretion from the lacrimal gland, or passive diffusion through the conjunctival vessels. The latter is theoretically enhanced when the blood-tear barrier is disrupted. In humans, this physiological feature could explain why the concentration-time profiles of cetirizine were similar in serum and tears in patients with allergic conjunctivitis.[157] In dogs, tear film corticosteroid levels were generally higher in conjunctivitis *vs.* control eyes following oral prednisone administration (up to +64%), although differences were not statistically significant.[160] The degree of conjunctival permeation is likely to vary among therapeutic drugs given differences in their physico-chemical properties.[275,276]

These findings highlight the importance of conducting pharmacological studies in clinically relevant preclinical species that are able to recapitulate leaky conjunctival vessels and elevated albumin levels in the tear film of clinical patients with ocular diseases. For topical drug administration, the authors recommend using an experimental model of blood-tear barrier breakdown (*eg.*, histamine-induced conjunctivitis or alkali burn models)[212,277] so that albumin and other relevant proteins are already present on the ocular surface at the time the drug mixes with the tear film.[229] For systemic drug administration, the authors suggest conducting a preliminary experiment to assess whether conjunctival inflammation affects tear film concentrations to a significant extent. If not, pharmacological assessment in healthy eyes should be sufficient. Incidentally, the physico-chemical properties of some drugs (*eg.*, size, lipophilicity, polar surface area)[275,276] may allow for high conjunctival permeation under normal conditions, thereby rendering differences between healthy *vs.* diseased eyes insignificant.

4.3.   Corneal injury in dogs: *in vivo* and *ex vivo* models

Corneal injury is common in human and veterinary patients – whether due to trauma, surgery, or other causes – and the resulting corneal scar remains one of the leading causes of blindness in animals and people worldwide.[278] Although small laboratory animal species are commonly used in corneal scarring research,[279] results derived from these models have several limitations. The corneal thickness is much smaller in rabbits and rodents compared to humans (**Table 1**). In addition to thin corneas, mice and rats have corneas that are much smaller in diameter compared to people; consequently, it is often difficult to isolate the central cornea when performing the experimental procedure (*eg.*, chemical burn) and the damage caused to surrounding limbal stem cells negatively impacts the wound healing process. Using the dog as an animal model is

therefore more appropriate, not only due to closer resemblances in ocular surface anatomy and physiology with humans, but also the relatively high prevalence of naturally-acquired corneal pathology in the canine species. In that regard, Gronkiewicz et al. recently developed a novel *in vivo* corneal fibrosis model in canines;[277] the authors induced corneal scarring with an alkali burn and investigated the ability of suberanilohydroxamic acid (SAHA) to inhibit fibrosis using this large animal model. The availability of such a model presents a clear opportunity for translational research (*ie.*, intact innervation, tear film, blood supply), although experimentally-induced corneal wounding (at risk of secondary infection) and subsequent corneal scar in dogs represent potential ethical challenges. As an alternative, other authors have established *ex vivo* canine corneal cultures that can be used to model wound healing and assess anti-fibrotic compounds,[280-282] or better understand the pathophysiology of herpesvirus in a virus-natural-host environment;[283] in that study, the authors established an air-liquid canine corneal organ culture model to study acute herpetic keratitis, showing important similarities in the response to CHV-1 to what has been described for HSV-1.[283]

5. Conclusions

"Considerable reservations may be felt about comparing results from rabbits with those from humans because of the differences between the physiology of tear flow and mixing and general anatomy. Nevertheless, the rabbit is the principal experimental animal in ophthalmology, so comparisons are needed".[284] Sadly, this quote published over 45 years ago is still representative of today's state of ophthalmic research. Rabbits and small laboratory rodents continue to be used primarily (if not exclusively) in most areas of ophthalmic research,[1] a concerning fact given the vast anatomical and physiological differences that exist with humans. Of note, such differences should not be regarded as merely 'weaknesses' for translational research, but rather evolutionary adaptations optimally suited to the environment and behavior of each species; for instance, rabbits likely developed a very stable tear film to limit intermittent blindness that occurs with each blink,[285] thereby reducing the risk of predation. Noteworthily, recent innovations have helped mitigate some of the drawbacks of traditional laboratory species – for instance providing manual blinking and supplementary tear flow in anesthetized rabbits,[286] or reverse engineering the ocular surface using human cells *in vitro*[287] – however the authors believe the complexity of the ocular surface and integrated lacrimal functional unit cannot be fully recreated without *in vivo* conditions in awake subjects.

The comparative work presented throughout this review provides evidence that dogs are best suited for translational research in ophthalmology. Unlike small laboratory animals, dogs share similar anatomical and physiological features to humans, similar environmental stressors and genetic variation, and a range of naturally occurring ophthalmic diseases that closely resemble clinical phenotypes in human patients. The resemblance between dogs and humans is particularly relevant in the field of ocular pharmacology, with notable similarities in blink rate, tear turnover rate (basal, reflex), volumetric capacity of the palpebral fissure, and other factors pertinent to drug diffusion (*eg.*, globe volume, corneal thickness); nonetheless, a few differences should be accounted for in comparative studies, such as the presence of a nictitating membrane, greater tear volume, larger corneal size and lower corneal elastic modulus in dogs. Similar to other fields of medicine, preclinical studies in ophthalmology could involve canine patients with spontaneous ocular diseases – many of which share striking resemblances with their human counterparts –

integrating the expertise of veterinarians, physicians and basic science researchers under the umbrella of the One Health Initiative.[6,288] Alternatively, or complementarily, preclinical animal work could be performed in laboratory dogs in whom ocular disease is experimentally-induced, making sure to account for the blood-tear barrier breakdown (noted in clinical patients with 'red eyes'). In all cases, tear fluid can be easily collected from canine eyes for various bioanalytical purposes, favoring Schirmer tear strips over other collection methods given the excellent safety profile and enhanced reliability in analyte quantification (*eg.*, proteins, drugs) provided by this method.

TABLES

Table 1. Comparative anatomy of the ocular surface and globe between humans, dogs and common laboratory species used in preclinical ophthalmic research.

		Human	Dog	Rabbit	Mouse	Rat
Lacrimal glands and nasolacrimal apparatus	Lacrimal glands	Lacrimal gland Accessory glands of Wolfring and Krause [20]	Lacrimal gland, Third eyelid gland [11,14]	Lacrimal gland, Third eyelid gland, Infraorbital (intraorbital), Accessory glands of Wolfring [10,11,22,23]	Infraorbital (extraorbital) [10,11,22]	Infraorbital (extraorbital) [10,11,22]
	Lacrimal glands volume (cm^3) †	0.59-0.61 [18]	0.14/ 0.1 [17]	– / 1.5 [289]	–	–
	Harderian gland	Absent [22]	Absent [11,16]	Present [11,22]	Present [11,22]	Present [11,22]
	Nasolacrimal drainage apparatus	Two puncta/canaliculi, no flexure [25,27]	Two puncta/canaliculi, 1 dorsal flexure [16,25,27]	Single punctum/canaliculus, two pronounced flexures [27,38,41]	Two puncta/canaliculi [25]	Two puncta/canaliculi [25]
Eyelids	Third eyelid	Absent [66]	Present [16,66]	Present [66]	Present [66]	Present [66]
	Palpebral fissure width (mm)	21.3-34.5 [290]	18.9-34.1 [40,291]	10-16 [38,41,42]	3.7-5 ‡ [43]	6-9 [45]
	Interpalpebral fissure area (cm^2)	1.8 [39]	2.2 § [290]	1.4 [39]	0.13 ‡ [43]	0.5 ¶ [45]
	Meibomian glands (/eyelid)	20-40 [39,292]	20-40 [16]	30-50 [38,39,41]	20 [293]	20-30 # [241]
Conjunctiva	Conjunctival fornix depth (mm) ††	15 [49]	–	20.36 [48]	–	–
	Conjunctival surface (cm^2)	17.65 [50]	–	13.3-18.48 ‡‡ [50]	–	–
	Conjunctiva/Cornea surface ratio	17.17 [50]	–	8.62-8.94 [50]	–	–
	Goblet cell spatial configuration	Individual [51]	Individual [52]	Individual [51]	Clusters [51]	Clusters [51]
	Goblet cell distribution	Highest in plica semilunaris and lower nasal fornix Low in bulbar conjunctiva [30,53,54,56]	Highest in third eyelid and lower nasal fornix Low in bulbar conjunctiva [52,55]	Highest in palpebral conjunctiva Relatively dense in bulbar conjunctiva [57-59]	–	Highest in fornix Low in bulbar conjunctiva [Kim 2019]

	Conjunctiva-associated lymphoid tissue	Present [61]	Present [16,61]	Present [61]	Only present in nictitating membrane [61,62]	Absent [61]
Cornea and Sclera	Corneal diameter, horizontal/vertical (mm)	11.8 / 11.2 [75,77]	13-17 / 12-16 [71,77]	13.4-15 / 13-14 [41,76,77]	2.3-2.6 / – [74]	2.0-6.8 / 2.0-6.7 [44,72,73]
	Corneal surface (cm^2)	1.04-1.3 [50]	1.2-2.1 [71,77]	1.55-2.03 [50]	0.04-0.05 [h] [74]	0.27-0.36 [§§] [72,73]
	Corneal thickness (μm)	505-563 [79]	497-594 [68,78]	354-407 [41,76,79,80]	90-137 [11,74,80]	159-170 [68,80]
	Corneal epithelial thickness (μm)	44-55 [79]	52-64 [71,78]	45-49 [79]	37-46 [74]	26-33 [85]
	Endothelial cell density (cells/mm^2)	2732 [90]	2818 [68]	3233 [68]	2875 [68]	2242 [68]
	Subbasal nerve plexus pattern	Whorl-like [67]	Whorl-like [67]	Horizontal [67]	Whorl-like [67]	Whorl-like [67]
	Corneal sensitivity (g/mm^2)	0.2-1.0 [2]	2.16-2.9 [92,95]	6.21-10 [2,92]	0.59 [93]	0.42-0.47 [91,94]
	Stiffness/elastic modulus (kPa) [¶]	16.2-33.1 [87,294]	1.3 [87]	1.1 [86]	25-40 [89]	6.2 [88]
	Scleral thickness at the limbus (mm)	0.50 [102]	0.80 [101]	0.29 [103]	0.05-0.06 [105]	< 0.1 [104]
Globe	Globe volume (mL)	5.7-6.0 [295]	5.0-5.8 [296]	2.3-2.9 [296]	0.014 [j] [297]	0.13 [##] [72]
	Anterior chamber (mL)	0.17-0.31 [66,77]	0.77 [101]	0.28-0.30 [41,66,77]	0.0044-0.007 [11,66,77]	0.0136-0.015 [11,66,77]
	Vitreous chamber (mL)	3.5-5.4 [65]	1.7-3.0 [101,296]	1.1-1.8 [66,296]	0.0053 [11,65]	0.013-0.054 [11,65]

– Information not available or not found

† Lacrimal gland (human), Lacrimal gland / TEL gland (dog), Lacrimal gland / Harderian gland (rabbit)

‡ Estimated based on clinical images[43]

§ Estimated based on clinical images[40]

¶ Calculated based on average palpebral fissure length[45]

Estimated based on clinical images[241]

†† Central upper conjunctival sac (from fornix to lid margin)

‡‡ Does not account for the nictitating membrane, surgically removed prior to the experiment

§§ Estimated from corneal radius, assuming a circular shape for the cornea in rodents[72]

¶¶ Whole cornea in rats, epithelium and anterior stroma in other species

Estimated from axial length, assuming a spherical shape for the globe

Table 2. Comparative physiology and characteristics of the ocular surface and tear film between humans, dogs and common laboratory species used in preclinical ophthalmic research.

		Human	Dog	Rabbit	Mouse	Rat
Ocular surface physiology	Blink rate (blinks/min)	8.5-17.6 [122-124]	14.2 [121]	0.05-0.19 [2,123,127]	0-4 [125,126]	2-5.3 [91,124]
	Volumetric capacity of the palpebral fissure (μL)	25-30 [109,136]	31.3 [40]	10-25 [†] [112,138]	≤ 5 [139,140]	≤ 20 [141]
	Ocular surface temperature (°C)	32.8-37.1 [298,299]	35.2 [300]	39.1 [298]	37.2 [298]	36.5 [298]
Tear film characteristics	Tear film thickness (μm)	2.3-11.5 [‡] [119]	15.1 [111]	6.5-18.4 [119]	7.4-21.1 [119,301]	2-12.6 [119,302]
	Lipid layer thickness (nm)	62-78 [123,303]	13-581 [121]	> 180 [123]	–	12 [301]
	Tear volume (μL)	7-12.4 [109,115]	65.3 [111]	1.9-7.5 [112,116]	0.06-0.2 [113,118]	4.6 [117]
	Basal tear turnover rate (%/min)	13.1-17.5 [109,110]	12.1 [111]	6.2-7.1 [112]	5.2 [113]	–
	Reflex tear turnover rate (%/min)	31.5-100 [109,115]	50 [111]	6.1-6.9 [§] [112]	–	–
	Evaporative rate (μm/min)	3.22 [304]	–	0.47 [127]	–	–
Tear film diagnostics [¶]	Schirmer tear test-1 (mm) [#]	10.0-18.6 [305,306]	18.1-24.3 [13,52,95,205,307,308]	4.6-7.6 [42,116,309]	–	5.6-9.4 [91,241]
	Phenol red thread test (mm/15s)	9-20 [310]	17.5-39.2 [95,205,307,308]	20.9-25.0 [42,309]	2.8-11.2 [125,244,311]	7.6 [312]
	Tear film breakup time (sec)	7.4-13.0 [305,306]	13.9-24.0 [13,52,205,307]	2-1788 [††] [313]	5-25 [125,129]	5.2-6.0 [241]
	Tear osmolarity [‡‡] (mOsm/L)	300.8 [202]	337.4-339.0 [205,314]	291.3 [315]	346.3-366.8 [310]	284.8 [312]
	Tear pH	7.83 [316]	8.05 [215]	8.2 [316]	7.59 [317]	–

– Information not available or not found

† Based on greater percent of drug lost at 1 min in rabbit eyes receiving 25 or 50 µL eyedrop *vs.* 5 or 10 µL eyedrop,[138] despite no changes in tear turnover rate for instilled volumes up to 50 µL[112]

‡ Excludes an outlier measurement of 41-46 µm[318]

§ Estimated from rabbit eyes receiving a large volume instilled eyedrop (25-50 µL)

¶ Reported values prioritized studies that did not use sedation or general anesthesia

Values reported in mm/5min (humans) or mm/min (all other species)

†† Large variability in studies' methodology, most using topical and/or general anesthesia prior to testing, resulting in non-physiologic and highly variable measurements for tear film break up time

‡‡ Measurements obtained with the same device (TearLab[TM], OcuSense Inc., San Diego, CA).

Table 3. Comparative composition of the major components in tear film between humans, dogs and common laboratory species used in preclinical ophthalmic research.

		Human	Dog	Rabbit	Mouse	Rat
Proteins	Lactoferrin [†]	Abundant 144,147	Low 146,147	Low 319,320	Low 147	Low 147
	Lysozyme	Abundant 144,147	Low 145,146	Low 190,319	Low 147	Low 147
	Lipocalin [‡]	Abundant 144,147,148	Low to moderate 146,147,149,150	Low 147,148,319,321	Low 147,198	Absent 147,148
	Lacritin	Moderate 144	Moderate 322	–	Absent 198	Present 323
	Secretory IgA	Moderate 144	Moderate 146,324,325	Present 22	Low 11	Moderate 326
	Serum albumin	Low 182	Low 146,150,212	Low 190,319	Present 198	–
	Peroxidase	Low 327	Low 146,325	Absent 190	–	Abundant 194
	Amylase	Low 327	–	Absent 190	–	Low 194
Mucins	MUC5AC	Present 11	Present 11,55	Present 11	Present 11	Present 11
	MUC1, MUC4, MUC16	MUC16 >> MUC1 > MUC4 153	MUC16 >> MUC1 > MUC4 153	MUC1 ≈ MUC4 ≈ MUC16 153	Present 328	Present 329
	Major O-glycans	Sialylated glycans 154	Fucosylated glycans 154	Fucosylated glycans 154	–	–
Lipids	Wax esters	Abundant 129,155	Abundant 129,155	Low 129	Abundant 129	Present 330
	Cholesterol	Low 129,155	Low 129,155	Low 129	Low 129	Abundant 330
	Cholesteryl esters	Abundant 129,155	Abundant 129,155	Low 129	Abundant 129	–
	DiHL	Low 129,155	Low 129,155	Low 129	Low 129	–
	DiHL esters	Low 129,155	Low 129,155	Abundant 129	Low 129	–
	DiAD	Low 129,155	Low 129,155	Abundant 129	Low 129	–
	OAHFA	Abundant 129,155	Abundant 129,155	Abundant 129	Abundant 129	–
	Chl-OAHFA	Moderate 129,155	Moderate 129,155	Low 129	Moderate 129	–

	Others [§]	Low 129,155	Low 129,155	Low 129	Low 129	–

– Information not available or not found

† Homologous iron-binding protein called transferrin is reported in dogs[146] and rabbits[319]

‡ Homologous proteins are reported in dogs (major canine allergen),[148-150] rabbits (lipophilin)[151] and rats (VEGr1)[148]

§ Triacylglycerol, squalene, ceramides, phospholipids and sphingomyelins.

FIGURES AND FIGURE LEGENDS

Figure 1. Graphical representation of the canine ocular surface and lacrimal functional unit.
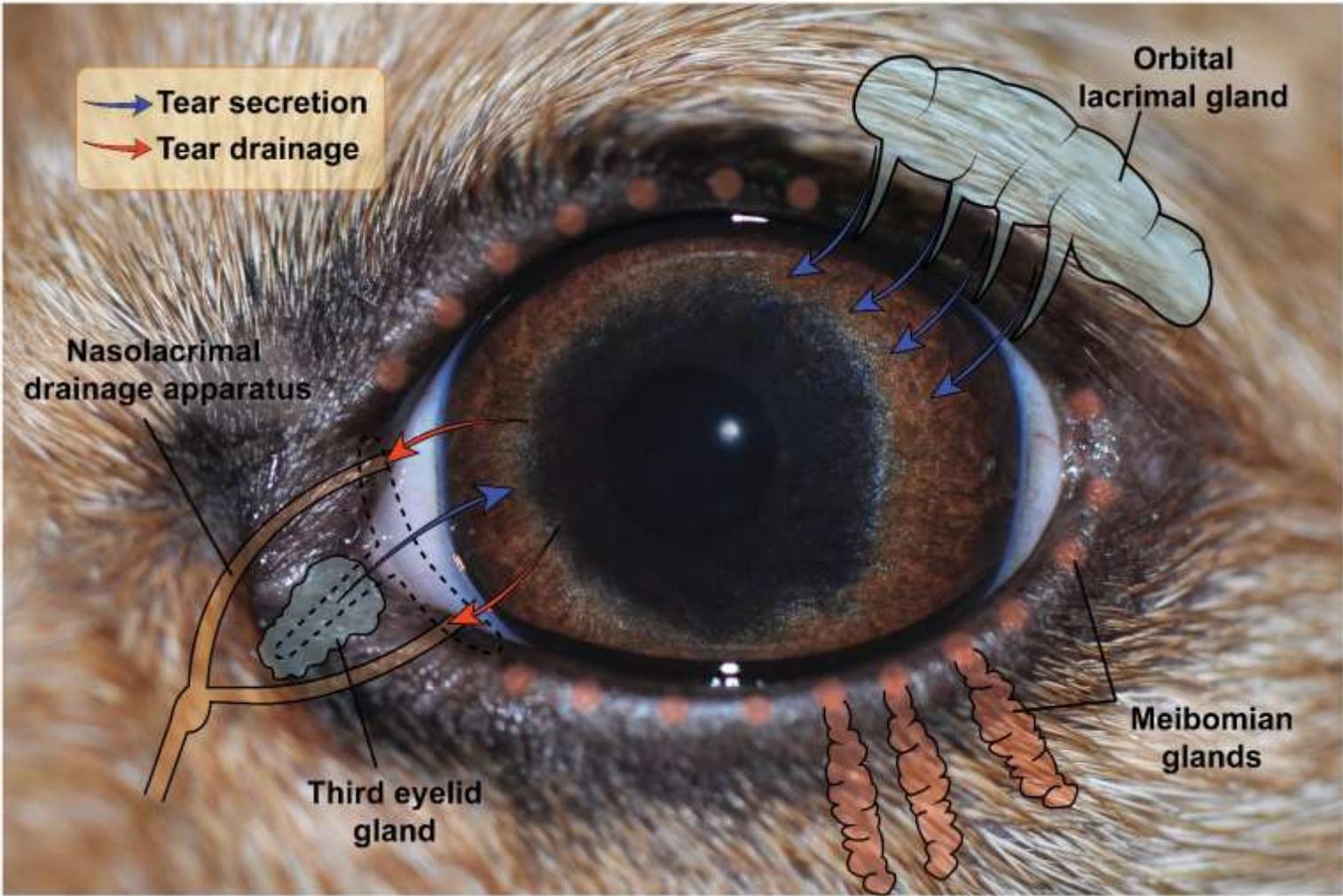
Figure 2. Diagram depicting the complexity of tear film dynamics and ocular surface physiology. Secretion of tear components, distribution of tears through blinking, and elimination through nasolacrimal drainage and evaporation must be precisely regulated to maintain homeostasis. Drug kinetics following topical eyedrop administration are impacted by key parameters highlighted in yellow, each being unique in different species. Adapted with permission from "Tsubota K, Tseng SCG, Nordlund ML. Anatomy and Physiology of the Ocular Surface In: Holland EJ,Mannis MJ, eds. Ocular Surface Disease Medical and Surgical Management. New York, NY: Springer New York, 2002;3-15".
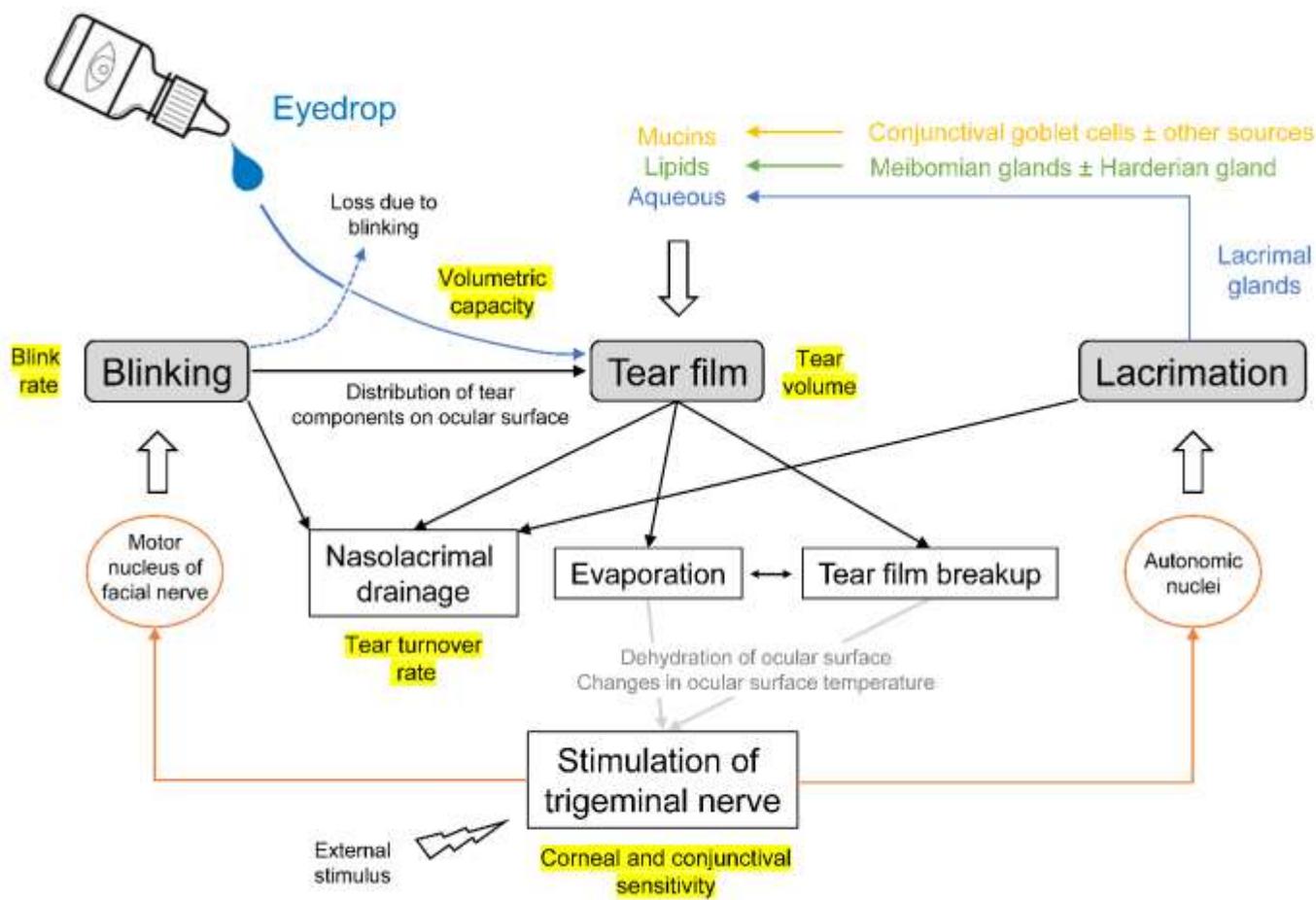
Figure 3. Tear collection in dogs using microcapillary glass tubes (a), Schirmer tear strips (b) or absorbent sponges (c).

Figure 4. Step-by-step protocol to exact tear fluid from Schirmer strips for analytical purposes, using a combination of centrifugation and solvent wash. Centrifugation of wetted Schirmer strip (containing tear sample and internal standard) retrieves tear fluid in a large tube, while subsequent solvent elution washes residual content from the absorbent filter paper.
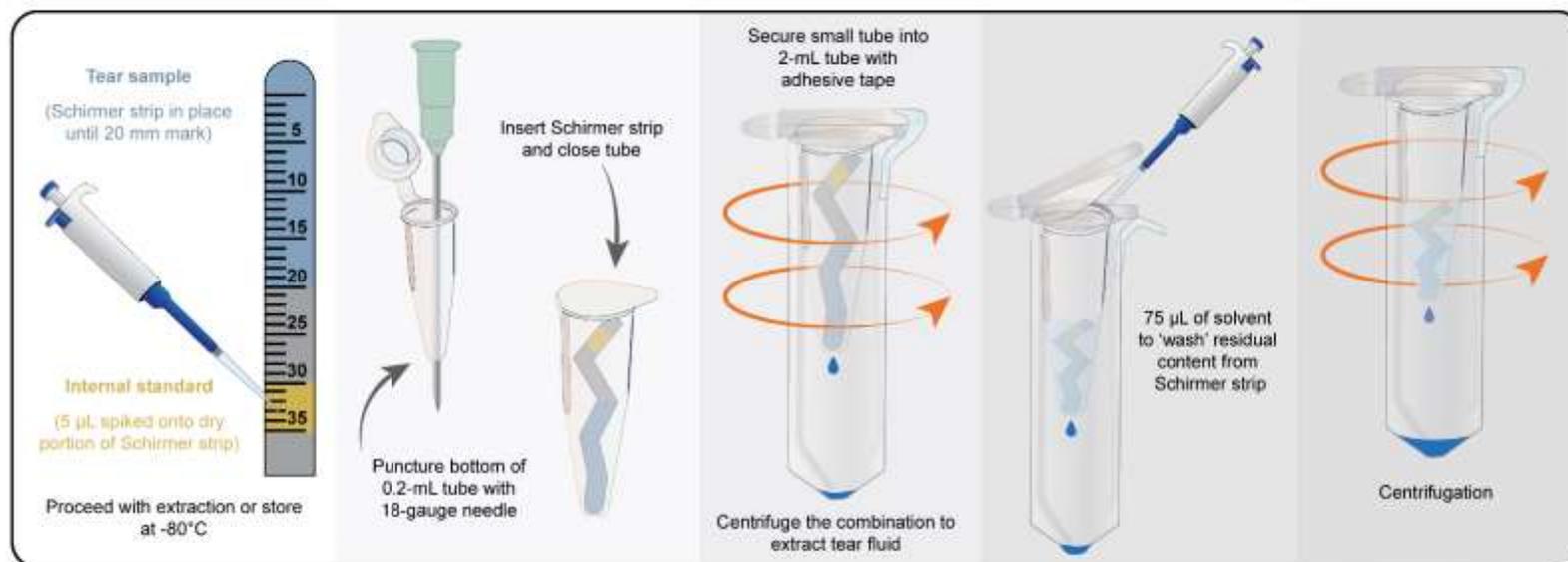
Figure 5. Representative clinical pictures of mild conjunctivitis (a), moderate conjunctivitis (b), and severe conjunctivitis (c) in dogs following topical administration of histamine at concentrations of 1 mg/mL, 10 mg/mL and 375 mg/mL, respectively. Reprinted from "Sebbag L, Allbaugh RA, Weaver A, et al. Histamine-Induced Conjunctivitis and Breakdown of Blood-Tear Barrier in Dogs: A Model for Ocular Pharmacology and Therapeutics. Front Pharmacol 2019;10:752".

Figure 6. Graphical representation of the blood-tear barrier in the canine eye. The barrier is intact in healthy eyes (left) but is disrupted in diseased eyes (right), enhancing the flow of compounds (*eg*, albumin, xenobiotics) between the tear film and the blood compartments. Breakdown of the blood-tear barrier can have important clinical implications such as enhanced systemic absorption from greater conjunctival vascular permeability, or reduction in ocular drug bioavailability due to drug-albumin interactions in the tear film.
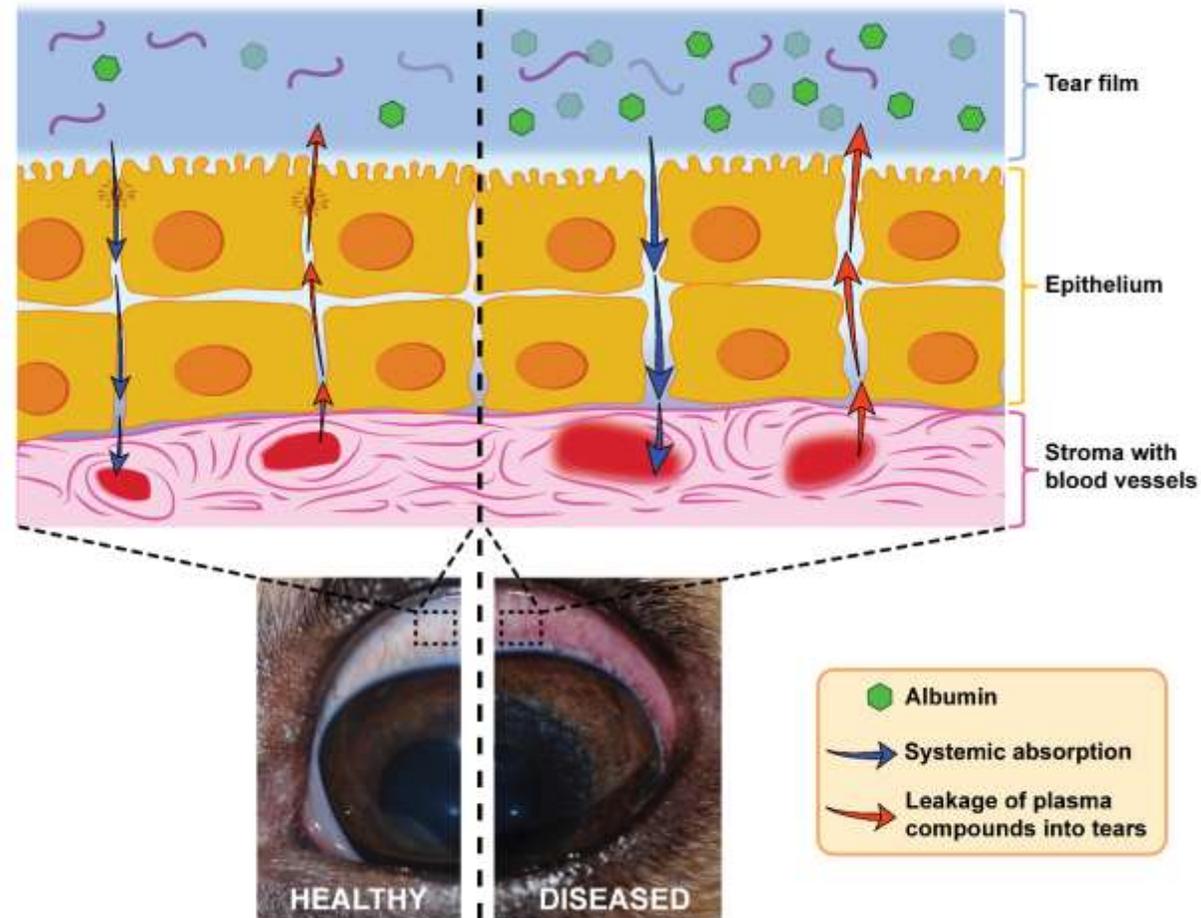
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Dr. Sebbag obtained his Veterinary Medical Degree from the National Veterinary School of Toulouse. He then completed a rotating internship at Kansas State University before pursuing a residency in Comparative Ophthalmology at the University of California-Davis. Dr. Sebbag is a Diplomate of the American College of Veterinary Ophthalmologists (ACVO), and currently holds a position as an Assistant Professor in the Department of Veterinary Clinical Sciences (Ophthalmology service) at Iowa State University, while completing a Ph.D in Biomedical Sciences focused on pharmacology and ocular disease models. Dr. Sebbag clinical and research interests include ocular surface diseases, tear film biology and innovations in drug delivery to the eye. He received multiple academic awards and published over 40 articles in select biomedical journals.

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Dr. Mochel obtained his Veterinary Medical Degree from the National Veterinary School of Alfort. He completed his Doctorate studying Neurosciences in collaboration with the College de France, and received the Silver Medal from Paris XII for his work. Dr. Mochel holds a MS in Pharmacology and Pharmacokinetics and is a Diplomate of the European College of Veterinary Pharmacology and Toxicology (ECVPT). He completed his Ph.D at Leiden University, with a focus on the mathematical modeling of the renin-angiotensin system for cardiovascular diseases. He is a founder of the Animal Health Modeling & Simulation Society which aims at promoting the dissemination of mathematical modeling in Veterinary Sciences. Dr. Mochel is an Associate Professor in the Department of Biomedical Sciences at Iowa State University (ISU) and the Chair of the Education and Residency Committee of the ECVPT. He is a Fellow of the American Academy of Veterinary Pharmacology and Therapeutics and the current Vice-President of the European Association of Veterinary Pharmacology and Toxicology. His research pertains to the analysis of clinical data obtained from spontaneous animal models of human diseases to bridge the knowledge gap between experimental models and patients. He has authored 70 peer-reviewed publications in select biomedical journals and was the 2019 recipient of the ISU Early Career Achievement in Research Award at the College of Veterinary Medicine.

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