Evaluation of the Precorneal Tear Film in Normal Rabbits Using a Novel Ocular Surface Analyzer

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Introduction

The precorneal tear film (**Fig. 1**) is comprised of three major layers: the outer lipid layer, middle aqueous layer, and inner membrane-bound and soluble mucin layer. Each layer is critical for maintenance of vision and ocular health. Therefore, handheld, non-contact instruments, such as the Ocular Surface Analyzer (OSA), which assess the tear film, the ocular surface, and interactions between the two are critical in the investigation of ocular surface disorders (OSDs). Compared to human medicine, there is little information regarding the tear film in veterinary patients, as well as in species such as rabbits used in ophthalmic drug development and OSD research. Therefore, the goals of this study were to evaluate, in rabbits, the OSA so as to establish normative data, evaluate repeatability of data, and compare some results with existing clinical methods of assessing tear film stability.

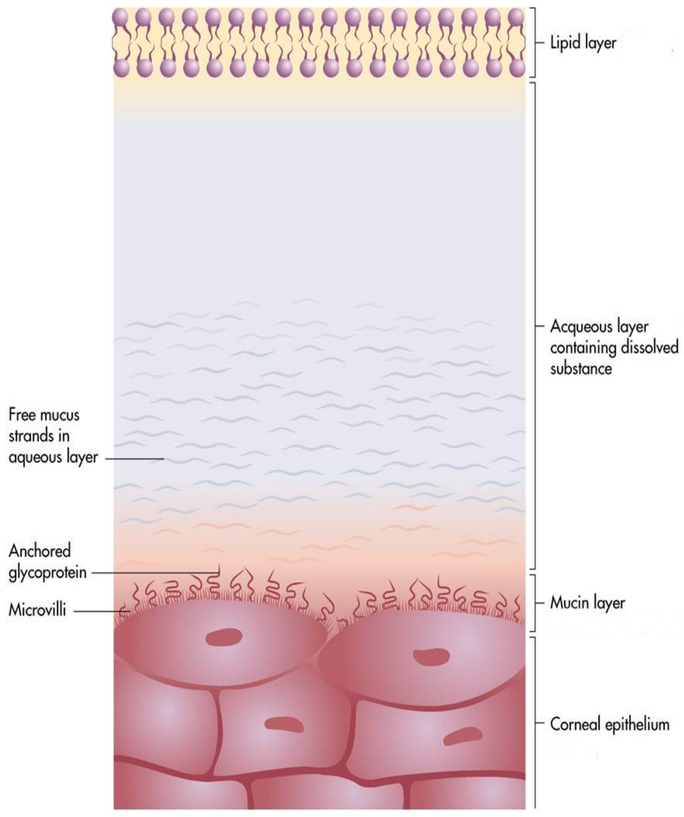

Figure 1[1]: Precorneal tear film anatomy, comprised of outer lipid layer, middle aqueous layer and inner mucin layer.

Methods

The OSA was used to evaluate 4 clinical parameters in 12 New Zealand White rabbits on 3 separate days: tear meniscus height (TMH), lipid layer thickness (LLT), non-invasive tear film break-up time (NIBUT), and Meibomian gland surface area (MGSA). All exams were performed according to manufacturer guidelines. Clinical ocular surface exams and tear film break-up time (TFBUT) were performed periodically by one author (SK). The TFBUT was assessed by instilling fluorescein on the surface of the eye and measuring time until "dark spots" appeared in the tear film over the dorsolateral cornea.[2] Rabbits with corneal defects were excluded from analysis of TMH, LLT, and MSGA, but did undergo TFBUT and NIBUT assessment. Ease of OSA use was evaluated subjectively by one author (KS). Normative data were calculated using a bootstrapping procedure performed on mean/median data collected across 3 days (Python Software Foundation). One way ANOVA was performed on data from each test day to evaluate measurement repeatability. Results of TFBUT and NIBUT were compared using a Wilcoxon paired test.

Results

Assessment of TMH (**Fig. 2A**) and LLT (**Fig. 2B**) using the OSA system were technically easy. Although assessment of NIBUT was technically difficult due to motion of the nictitating membrane and sometimes the globe or rabbit, break-up was still visualized (**Fig. 2C**). Visualization of Meibomian glands and calculation of MGSA was difficult due to poor contrast of the Meibomian glands against the eyelid (**Fig. 2D**) and difficulty everting the lower eyelid. Mean ± SEM of TMH and NIBUT were 0.208 ± 0.008 mm (**Fig. 3A**) and 63.8 ± 10.5 seconds (**Fig. 3B**), respectively. Repeatability of TMH (P = 0.308) and NIBUT (P = 0.53) over 3 days was good. Considering all rabbits (with and without corneal defects), NIBUT (48.6 ± 5.32 seconds) was significantly greater than TFBUT (23.4 ± 3.19 seconds; P = 0.0078; **Fig. 3C**). This difference was also seen when only rabbits without corneal defects were assessed (NIBUT = 51.0 ± 9.73 seconds; TFBUT = 26.7 ± 3.90 seconds; P = 0.001; **Fig. 3D**). However, a significant difference was no longer detected when only rabbits with corneal defects were assessed (NIBUT = 30.7 ± 9.47 seconds; TFBUT = 18.8 ± 2.11 seconds; P = 0.125; **Fig. 3E**). The OSA software estimated the rabbit LLT as ~30 nm. Assessment of MGSA revealed median ± SE Meibomian gland loss in the upper and lower lids as 11.5 ± 4.03% and 32.5 ± 7.82%, respectively.

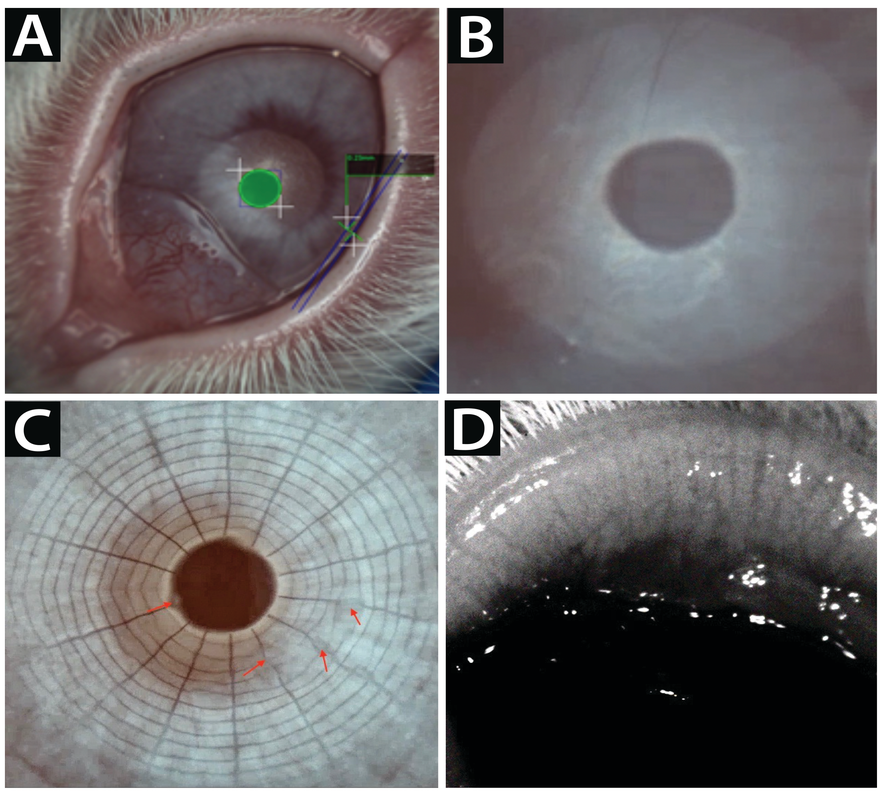

Figure 2: Representative images of the ocular surface of a rabbit obtained using the ocular surface analyzer. **A)** Tear meniscus height (within blue lines). The green circle sets the working distance for analysis. **B)** Lipid layer thickness. Note the simple wave patterns and lack of coloration. **C)** Non-invasive break-up time. Tear film break-up manifests as a disturbance of the grid lines (red arrows). **D)** Meibography of the upper eyelid.

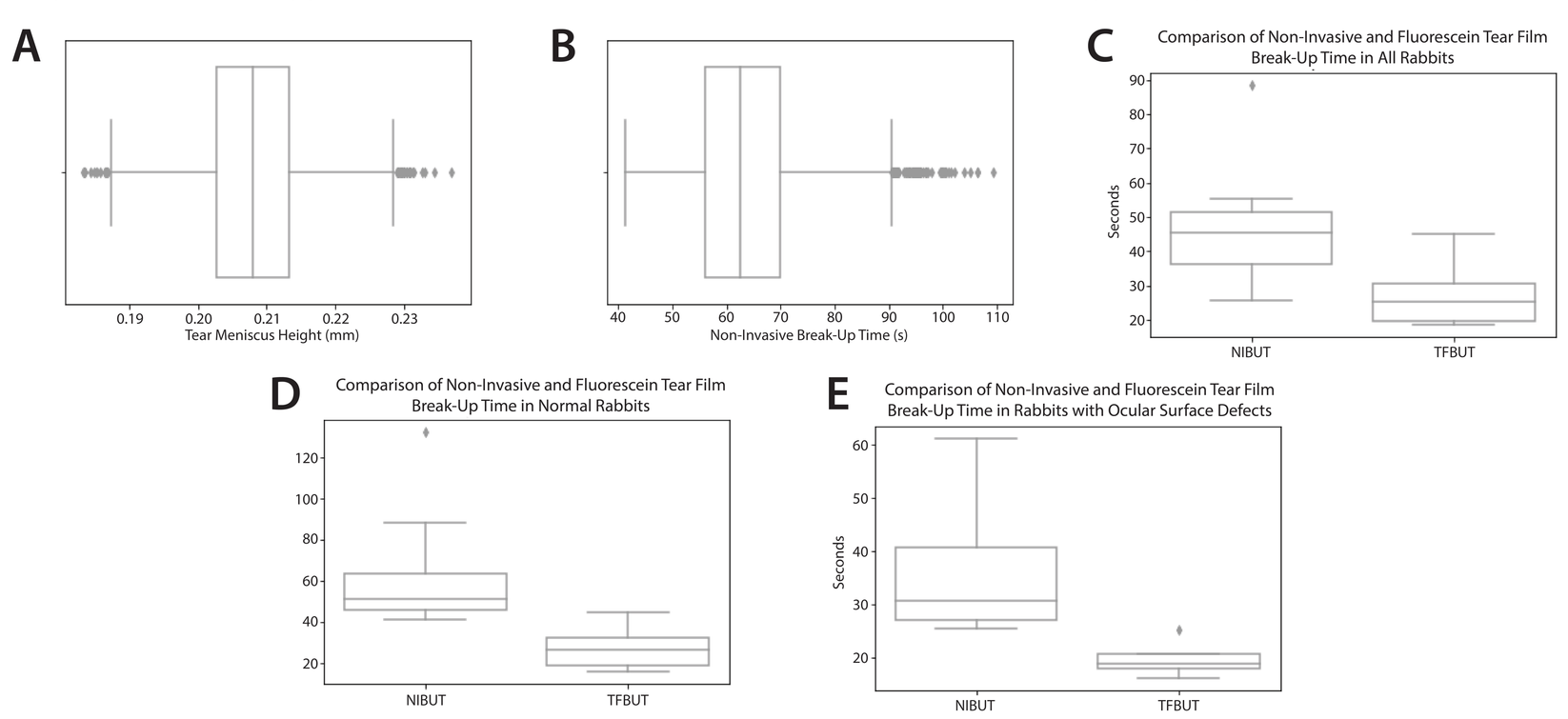

Figure 3: Distribution of various ocular surface parameters as measured using the ocular surface analyzer in New Zealand White rabbits. **A)** Tear meniscus height measurements (mm) collected in over 3 days. **B)** Non-invasive break-up time (seconds) collected over 3 days. **C)** Comparison of non-invasive break-up time (seconds) and tear-film break-up time (seconds) in rabbits without corneal defects. **D)** Comparison of non-invasive break-up time and tear-film break-up time (seconds) in rabbits with and without corneal defects. **E)** Comparison of non-invasive break-up time and tear-film break-up time (seconds) in rabbits with corneal defects.

Conclusion

The human equivalent of the OSA provides a thorough analysis of the ocular surface that can be useful in patients with OSDs. However, the present study identified some limitations in applying the canine instrument to rabbits – a species used frequently in OSD research and drug testing. These limitations were associated with software, hardware, or ocular anatomy. For example, the OSA estimated LLT as 30 nm which is thinner than has been calculated using other methods (~180 nm).[3] This may be because the patterns generated by the lipid layer of dog tears differ from those generated by rabbit tears and a new scaling method should be developed. Meibography was also challenging in the rabbits we examined due to reduced contrast of the glands against the eyelids. It seems likely that the decreased contrast was because albino rabbits were used, and this may have resulted in the OSA software failing to detect a number of Meibomian glands and calculating notable Meibomian gland loss. It is also possible that the rabbits chosen did have notable Meibomian gland loss and histologic assessment would have been necessary to confirm this. The shorter TFBUT compared to NIBUT in patients without corneal defects confirms earlier reports that the application of fluorescein and/or eyelid manipulation required for TFBUT may alter tear film dynamics and suggests that the NIBUT may be more reliable. Regardless of the issues with identification of lipid abnormalities, the OSA system was useful in measuring the tear film stability through NIBUT.

References:
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