

The Application of Strip Meniscometry to the Evaluation of Tear Volume in Mice

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PURPOSE. Strip meniscometry quantitatively measures the volume of tears in the tear meniscus and has been reported to diagnose dry eyes in clinical settings conveniently, easily, and rapidly. In this study, we used a modified strip meniscometry to assess the applicability of measuring the tear volume in mice in experimental settings.

METHODS. Dry eye was induced in 11 9-week-old C57BL/6J wild-type male mice (11 right eyes) by exposing them to an air fan inside a small compartment for 5 hours for 2 consecutive days. Tear function tests, including the SMTube for mice (SMTM) for tear volume evaluation, break-up time, fluorescein staining score, and lissamine green staining score, were performed. The correlation between SMTM and other tear function parameters was assessed.

RESULTS. The mean SMTM value was 3.89 ± 0.603 mm before and 3.09 ± 0.625 mm after dry environment exposure ($P = 0.0078^*$). The Spearman's correlation by rank test showed a strong positive correlation between SMTM and tear film break-up time and a strong linear negative correlation with fluorescein and lissamine green values.

CONCLUSIONS. The SMTM was capable of rapidly measuring the minimum tear volume in mice and correlated well with tear function parameters and appears to be a promising new modality in the evaluation of dry eyes in mice.

Keywords: SMTube, strip meniscometry, mouse, dry eye

Strip meniscometry (SM), reported by Dogru et al.¹ in 2006, is a new noninvasive device for quantifying the tear volume in the tear meniscus (TM) in humans. The test has been shown to have a strong correlation with the Schirmer test, tear film break-up time (BUT), and ocular surface vital staining scores.² It has been used not only for the diagnosis of dry eye^{3,4} but also for the evaluation of treatment effectiveness of punctal occlusion and punctal plugs,^{5,6} as well as in routine health examinations.⁷ Its applicability has been reported in healthy dogs, cats, and rabbits without dry eyes.⁸

Mice are often used in dry eye research because of their abundant availability, low maintenance costs, and the ability to genetically modify their strains, which enables the study of dry eyes associated with different disease states.⁹ The cotton thread test has been traditionally used to measure the tear quantity in mice, which is a well-known means of tear volume assessment.^{10,11} However, the presence of a big standard deviation and reproducibility of testing are the problematic issues with this methodology. In this study, we examined the usefulness of SMTube in tear volume measurement in an environmental stress exposure dry eye mouse model.

MATERIALS AND METHODS

In total, 11 right eyes of 11 nine-week-old C57BL/6J male mice were used in the present investigation. All examinations adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

The Environmental Dry Eye Stress (EDES) Mouse Model

Each mouse was separately placed in a small compartment, and continuous air flow (4 m/s) was blown using an air fan placed 5 cm away from the mouse for 5 consecutive hours for 2 days to induce dry eye. This EDES mouse model was originally developed as a rat dry eye model in our laboratory¹² and has subsequently been applied in mice.¹³ During the experiment, the room temperature was maintained at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and humidity at $25\% \pm 2\%$.

The SMTube Testing (SMTM)

The improved version of the SMTM has certain similar aspects to the original version of the SMTube product. For instance, one single SMTM strip is designed to perform the testing in both eyes of a patient and the strip possesses a tubular structure to induce the capillary action, which aids in tear absorption. A comparison of the appearance between the SMTube and the SMTM strips is illustrated in (Fig. 1).

An SMTM strip is formed with a three-layered structure. The top layer and the bottom layer comprise polyurethane tape and polyester tape, respectively, while the middle layer comprises polyurethane backing that forms a ditch along the longitudinal dimension (Fig. 2). This component has a tubular vacuum structure, which induces capillary action along the tear absorption path when the tear fluid touches the tip of the strip. The tear absorption path is filled with an absorber made of nonwoven fabric. This material is composed of polyethylene





FIGURE 1. A comparison between the original SMTube strip and the SMTM strip developed devoted to the use in mice. Differences in the form of the strips: SMTube (*top*) and SMTM (*bottom*).

terephthalate and has densely stacked fibers that are approximately 5 μm in thickness (Fig. 2), facilitating a smooth and uniform absorption of the tears. Another difference of the SMTM strip is the width of the tear absorption path: it has been modified from 0.9 mm to 0.4 mm. This change was made in an attempt to improve the sensitivity to an increment in small tear volume range.

SMTM Testing

In human testing, immediately after a couple of blinks, the tip of the SMTube is gently immersed into the inferior TM without touching the eyelid or the ocular surface and statically held at the same position for 5 seconds.³ The blue-stained length of the tear-absorbing column is then swiftly read as the SMTube score.³

Similarly, in mice, SMTM was gently immersed into the inferior TM of the eye of a mouse with touch to the eyelid and ocular surface (Fig. 3). The measurement time was set to 5 seconds, which is the same time used in humans. An electronic metronome was used for the strict measurement of the testing duration. Measurements were performed prior to and 1 hour after wind exposure. The SMTM measurement and all the following tests were performed by the same examiner.

Tear Ocular Surface Vital Staining Examinations and BUT Measurement

Vital staining examinations were performed using 0.5% sodium fluorescein (FS) and 1% lissamine green (LG) dye. For observation of the anterior ocular segment, a handheld slit lamp (an SL-15; Kowa, Tokyo, Japan) was used, and FS staining was evaluated using cobalt blue light. First, 2 μl of FS was instilled in the eyes by using a micropipette, and the BUT was measured. The time between blinking until the appearance of the first corneal black spot was measured three times, and the average value was obtained as BUT. Vital staining scores of FS and LG were obtained using a grading system of 0 to 3 points for superior, central, and inferior corneal areas (minimum of 0 to a maximum of 9 points). The measurement was performed before and 1 hour after air-blowing.

Statistical Analyses

Comparison of Examination Values Before and After Environmental Stress Exposure. The Wilcoxon matched-pairs signed rank test was used for statistical analysis, and $P < 0.05$ was considered as statistically significant.

Correlation Between SMTM and BUT, FS, and LG. The Spearman rank correlation coefficient was calculated to evaluate the correlation between each pair of examinations.

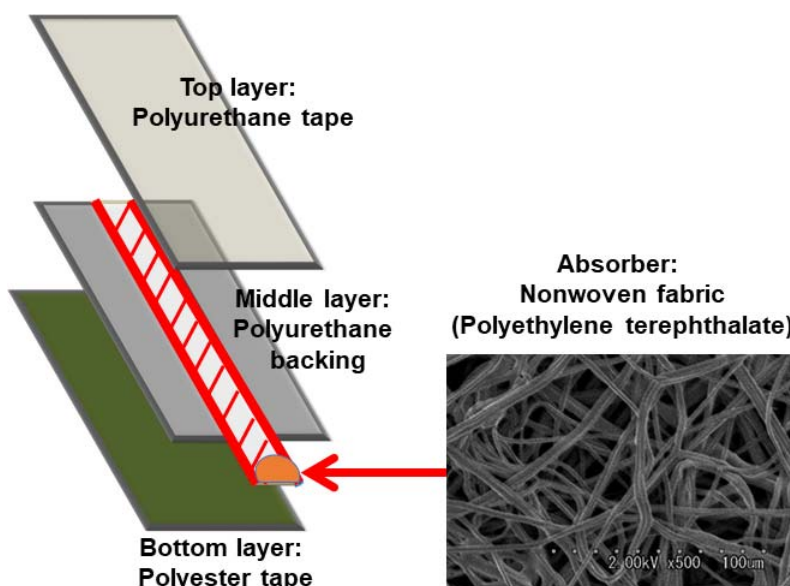


FIGURE 2. The structure of the SMTM. SMTM principally follows the original three-layered configuration forming a tubular absorption path. The *top layer*, *middle layer*, and the *bottom layer* consist of polyurethane tape, polyurethane backing, and polyester tape, respectively. The central ditch filled with the absorber functions as the tear fluid path. The material of absorber is a nonwoven fabric composed of polyethylene terephthalate, and its scanning electron microscopy image is inset at the *bottom right*. Dense and thin (approximately 5 μm) fibers facilitate smooth and uniform absorption of tear.



FIGURE 3. The SMTM testing. The examiner gently immersed the SMTM into the inferior TM of the eye of a mouse.

RESULTS

SMTM Testing

The SMTM measurement values (mean \pm SD) were 3.89 ± 0.603 mm before dry environmental stress exposure and 3.09 ± 0.625 mm after stress, showing a significant difference between the values before and after stress exposure ($P = 0.0078^*$; Fig. 4).

Tear Ocular Surface Vital Staining Examinations and BUT Measurement

The staining scores of FS (mean \pm SD) were 0.364 ± 0.505 and 4.09 ± 1.14 points before and after environmental stress exposure, respectively, showing a significant difference between the pre- and poststress exposure values ($P = 0.001^*$; Fig. 4).

The staining scores of LG (mean \pm SD) were 0.727 ± 0.647 and 4.55 ± 0.934 points after environmental stress exposure, showing a significant difference between the pre- and poststress exposure values ($P = 0.001^*$; Fig. 4).

The measurement values of BUT (mean \pm SD) were 7.73 ± 1.27 and 5.09 ± 0.701 seconds after environmental stress exposure, showing a significant difference between the pre- and poststress exposure values ($P = 0.039^*$; Fig. 4).

Correlation of SMTM With BUT, FS, and LG. The results of Spearman correlation by the rank test are shown in Figure 5. The correlation coefficients of SMTM with tear function

parameters were $r = 0.46$ for BUT ($P = 0.031^*$), $r = -0.84$ for FS ($P = 0.042^*$), and $r = -0.81$ for LG ($P = 0.066$).

DISCUSSION

Preclinical experiments are essential to fully understand the pathology of diseases and to aid in the development of therapeutics. Although various preclinical models of dry eyes are routinely used (i.e., dogs, rabbits, and rats are used), mice are the most common species used to study dry eyes owing to their abundance, ease of maintenance, and the presence of a wide variety of genetically altered strains and specific reagents for study.^{9,14} The analysis of mouse tears is useful for developing diagnosis and treatment strategies for human diseases. Tear function tests and ocular surface examinations used in mouse experiments briefly include the vital staining scores to evaluate ocular surface epithelial damage and BUT to study the tear stability. The traditional cotton thread method has been considered to be useful for tear quantity measurement, which can be applied to small eyes of mice with some irritation. A previous investigation demonstrated that the time required for measurement using this method is between 20 and 60 seconds.^{10,11,13} Therefore, although the cotton thread method is a tool for tear quantity measurement, the procedure is technically difficult because it requires holding the thread on the ocular surface in the lateral canthus for several tens of seconds by using only a pair of forceps. Moreover, the cotton thread testing has been excluded from the Japanese dry eye diagnostic criteria due to problems involved with reproducibility and the presence of wide standard deviations. In this study, we measured the TM volume before and after inducing dry eye in an already established environmental stress dry eye mouse model by using SMTM that was improved for use in mice and evaluated its usefulness as a parameter of ocular surface examination in mouse experiments.

The testing correlated well with other tear function parameters, including BUT and vital staining scores. The SMTM appears to have advantages in relation to the stability of the material while holding, the overall convenience of the technique, and short measurement time were considered to be an appropriate tear measurement tool for mice based on our results. One possible disadvantage is that because the mouse eyes are small the tips of the strips sometimes inevitably touch the lids and conjunctiva, which might induce reflex tearing.

The experiments in this study were performed by a single examiner. Another study performed under a different protocol, in which three examiners performed tear measurement on the same mice ($n = 8$) using SMTM, showed no significant

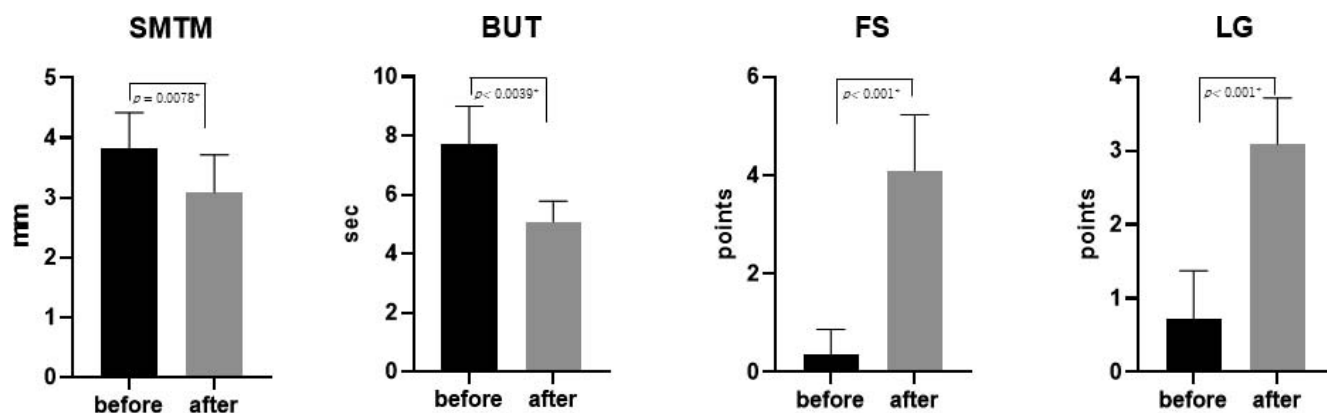


FIGURE 4. Comparison of SMTube, BUT, FS, and LG before and after exposing to an air fan. Statistically significant differences were found in results between before and after wind exposure by the Wilcoxon matched-pairs signed rank test. Asterisks denote statistical significance.

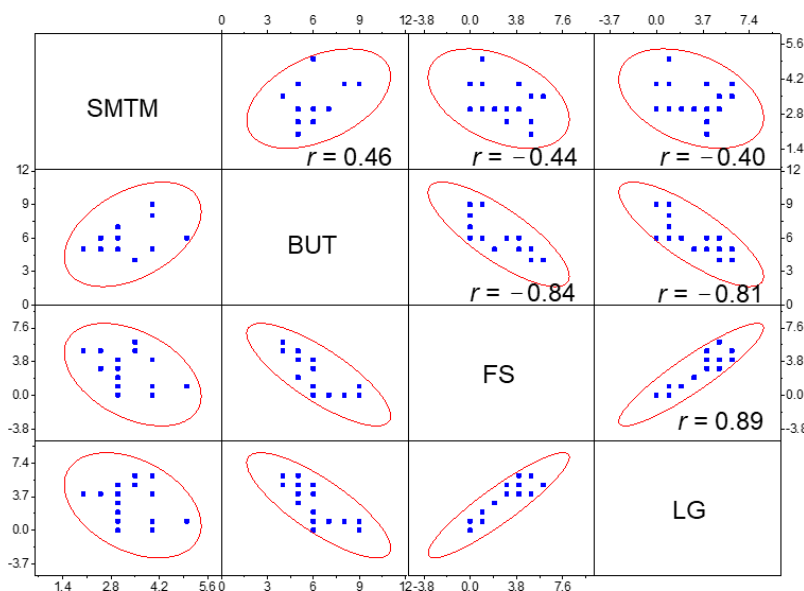


FIGURE 5. Correlation among SMTM for mice and dry eye disease parameters Spearman's correlation by rank test. X- and y-axis units: SMTM, mm; BUT, seconds; FS, points; LG, points.

differences between the examiners (Friedman test, $P = 0.578$) (data not shown). The SMTM enabled rapid and efficient absorption of the tears in the meniscus and evaluation of the tear volume in mice and appears to be a promising tool for dry eye research in mice.

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